DISSERTATION TITLED

"COMPARISON OF ANTIBODY TITERS BETWEEN THIRD AND FOURTH IM [INTRAMUSCULAR] DOSE OF ANTI RABIES VACCINATION FOLLOWING POST EXPOSURE PROPHYLAXIS WITH ANTI-RABIES IMMUNOGLOBULIN, AND KAP [KNOWLEDGE ATTITUTE AND PRACTICE] AMONG PATIENTS COMING FOR POST EXPOSURE PROPHYLAXIS OF DOG BITE"

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CERTIFICATE

This is to certify that the dissertation entitled **"COMPARISON OF ANTIBODY TITERS BETWEEN THIRD AND FOURTH IM [INTRAMUSCULAR] DOSE OF ANTI RABIES VACCINATION FOLLOWING POST EXPOSURE PROPHYLAXIS WITH ANTI-RABIES IMMUNOGLOBULIN, AND KAP [KNOWLEDGE ATTITUTE AND PRACTICE] AMONG PATIENTS COMING FOR POST EXPOSURE PROPHYLAXIS OF DOG BITE"** is a bonafide work done by **Dr. A.ABINANTHA RAJU**, Post Graduate Student, Institute of Internal Medicine, Madras Medical College, Chennai-3, during March 2014 to August 2014 in partial fulfillment of the University Rules and Regulations for the award of MD Branch – I General Medicine, under our guidance and supervision, during the academic year 2012 - 2015.

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DECLARATION

I solemnly declare that the dissertation entitled **"COMPARISON OF ANTIBODY TITERS BETWEEN THIRD AND FOURTH IM [INTRAMUSCULAR] DOSE OF ANTI RABIES VACCINATION FOLLOWING POST EXPOSURE PROPHYLAXIS WITH ANTI-RABIES IMMUNOGLOBULIN, AND KAP [KNOWLEDGE ATTITUTE AND PRACTICE] AMONG PATIENTS COMING FOR POST EXPOSURE PROPHYLAXIS OF DOG BITE"** is done by me at Madras Medical College, Chennai-3 during March 2014 TO August 2014 under the guidance and supervision of **Prof. K.S.CHENTHIL, M.D.,** to be submitted to The Tamilnadu Dr. M.G.R Medical University towards the partial fulfillment of requirements for the award of M.D. DEGREE IN GENERAL MEDICINE BRANCH-I.

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ABBREVIATIONS

CCV	-	CELL CULTURE VACCINE
CDC	-	CENTER FOR DISEASE CONTROL
CNS	-	CENTRAL NERVOUS SYSTEM
EEG	-	ELECTRO ENCEPHALOGRAM
ELISA	-	ENZYME LINKED IMMUNOSORBENT ASSY
FAT	-	FLOURESCENT ANTIBODY TEST
FAVN	-	FLOURESCENT ANTIBODY VIRUS NEUTRALISATION
HDCV	-	HUMAN DIPLOID CELL VACCINE
HRIG	-	HUMAN RABIES IMMUNOGLOBULIN
ID	-	INTRADERMAL
IM	-	INTRAMUSCULAR
MRI	-	MAGNETIC RESONANCE IMAGING
NTV	-	NERVE TISSUE VACCINE
PCECV	-	PURIFIED CHICK EMBRYO CELL VACCINE
PDECV	-	PURIFIED DUCK EMBRYO CELL VACCINE
PCR	-	POLYMERASE CHAIN REACTION

PEP	-	POST EXPOSURE PROPHYLAXSIS
PVRV	-	PURIFIED VERO CLL VACCINE
RFFIT	-	RAPID FLOURESCENT FOCUS INHIBITION TEST
RNA	-	RIBONUCLEIC ACID
RT- PCR	-	REVERSE TRANSCRIPTASE POYMERASE CHAIN REACTION
WHO	-	WORLD HEALTH ORGANISATION

CONTENTS

COMPARISON OF ANTIBODY TITERS BETWEEN THIRD AND FOURTH IM [INTRAMUSCULAR] DOSE OF ANTI RABIES VACCINATION FOLLOWING POST EXPOSURE PROPHYLAXIS WITH ANTI-RABIES IMMUNOGLOBULIN, AND KAP [KNOWLEDGE ATTITUTE AND PRACTICE] AMONG PATIENTS COMING FOR POST EXPOSURE PROPHYLAXIS OF DOG BITE

ABSTRACT:

Rabies is a fatal encephalitis transmitted to humans by the bite of rabid animals. Which is the most common mode of transmission in our country. Right now the important focus is on the post exposure prophylaxis since no effective treatment options are available till date. Over the course of time post exposure prophylaxis has been changing mainly because of the production of more potent vaccines and better understanding in the field of immune response for vaccination against rabies.

In our study we try to find out the effectiveness between the third and fourth dose of anti-rabies vaccination used in post exposure prophylaxis when given via intramuscular route. RAPID FLOURESCENT FOCUS INHIBITION TEST was used to measure the level of antibodies after seven days of each corresponding doses of

vaccination. If the adequate levels of antibodies were reached after the third dose and also if no significant increase in antibody titer after the fourth dose, the need for the fourth dose in the schedule can be eliminated.

In our study we also try to assess the knowledge, attitude and practice regarding dog bite among the patients coming for post exposure prophylaxis.

Conclusion:

In our study we found that the adequate levels of antibodies are reached even after the third dose itself, but the increase in antibody titer was highly significant when compared to the third dose. Therefore fourth dose has to be given as per the recommended schedule.

In our study we found that the knowledge levels regarding dog bites and rabies are adequate but the attitude and practices are not adequate and needs to be improved.

Keywords:

Dog bite, rabies, post exposure prophylaxis, intradermal route, intramuscular route, antibody titers, rapid fluorescent focus inhibition, knowledge, attitude, practices.

INTRODUCTION

Rabies is a fatal encephalitis caused by rabies virus. It is acquired through the bite of rabid animal as well as via contamination of mucus membrane with saliva of rabid animal. The other modes of transmission of rabies virus do not play a predominant role in the rabies transmission in our country.

Once diagnosed rabies is nearly always fatal. Because till today there was no effective management exist for human rabies. Mortality is always nearly 100%. Even in very rare cases of recovery, severe neurological sequalae persist. This calls for the effective post exposure management prevention of rabies.

When the post exposure prophylaxis is properly administered the rabies is completely preventable. Post exposure prophylaxis consist of wound management, active immunization against rabies and passive immunization in the form of human or equine immunoglobulin if needed.

Current guidelines are recommended by both world health organization and center for disease control. Post exposure prophylaxis by means of active immunization is given via intramuscular or intradermal route.

Till 2010 both regimen has a total duration of 28 days. Center for disease control in June 2010 came up with the recommendation of reduced dose of post exposure intra muscular active immunization. There by reducing the duration of active immunization for post exposure prophylaxis has been reduced to 14 days. This improves the patient compliance when compared to 28 days

In India both intramuscular and intradermal route was approved for use. But intradermal route is widely followed in government run institutions because of the economic concerns. The intradermal route requires less vaccine dose. The patient's compliance is decreased when the number of days required for completion of active immunization.

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Recently many studies demonstrate that neutralizing antibody levels against rabies virus reaches above the protective levels required after 4^{th} intramuscular dose. The test used for this measurement is Rapid Fluorescent Focus Inhibition Test [RFFIT], which is the authorized for this purpose by world health organization. Now studies are coming up with results showing that even after the 3^{rd} intramuscular dose the protective antibody levels are attained and these are maintained during follow up also. These leads to the reduced vaccine recommendation by center for disease control. Even though

the vaccination dose has been changing over time, the dose of anti-rabies immunoglobulin required for passive immunization remains unchanged.

In our study we try to assess the antibody response between the 3rd and 4th dose of intramuscular anti-rabies vaccination. So unless significant titer increase is noted after the fourth dose, the need for the fourth dose can be eliminated. We also try to assess the knowledge attitude and practice regarding dog bite in the dog bitten individuals.

AIM AND OBJECTIVES

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- To study the effectiveness of third dose of ARV with fourth IM dose to determine whether the fourth dose is really needed or not?

- To assess the knowledge attitude and practice among patients receiving post exposure prophylaxis

REVIEW OF LITERATURE

REVIEW OF LITERATURE

ETYMOLOGY:

The term rabies means “MADNESS” in Latin .it can also be related to Sanskrit word “RABHAS” means “TO DO VIOLENCE”. While the Greek word “LYSSA” means “VIOLENT” this forms the basis of the genus name Lyssavirus.

HISTORICAL SIGNIFICANCE:

Rabies has been known to mankind since around 2000 B.C. the first written evidences can be found in Mesopotamian Code of Eshnuna 1930 B.C. one of the earlier methods of treatment consists of wound cauterization by applying heated “St Hubert’s key”

A woodcut from the Middle Ages showing a rabid dog.

RABIES VIRUS:

Family : Rhabdiviridae

Order : Mononeglavirus

Genus : Lyssavirus

The rabies virus is bullet-shaped, the dimension of the viruses are around 180 nm in length and around 75 nm in diameter. The virus contains a helical nucleocapsid of 30–35coils with a length unwound between 4.2 and 4.6µm. The nucleocapsid enclosed in a 7.5–10-nm-thick lipoprotein envelope; from this envelope, glycoprotein (G protein) spikes project out another 10 nm. Except the blunt end these glycoprotein covers the entire surface of viruses. The viral genome is a single negative strand of RNA weighing 4.6 MDa, and contains a sequence of 11932 nucleotides. This RNAencodes five genes, named N, NS (or M1), M (or M), G, and L. The Nnucleoprotein binds to the RNA and is probably involved in the control of viral RNA replication; it is a potential immunogen. The purpose of the NS phosphoprotein is less clear, but it may control the L protein, which is the polymerase for replication. The M (matrix) protein is the major structural protein and is probably located between the nucleocapsid and lipoprotein envelope; its role is uncertain. The G protein is involved in cell surface reception, and it is the only antigen that induces virus-neutralizing antibodies. Variability in this protein accounts for serotypic differences among

lyssaviruses, and mutations at arginine 333cause loss of virulence (substitution with glutamine or isoleucine). This arginine residue is required for G protein mediated fusion of the viral envelope with neurons. Molecular modifications of the G protein can increase its antigenicity and may aid in the search for better vaccines.

The nicotinic acetylcholine receptor is an important viral binding site, but it is probably not the only one. Once bound to the receptor, receptor mediated endocytosis allows the virus to enter the neuron. This forms a coated pit, which then fuses with a lysosome, where enzymes release the nucleocapsid into the cytosol. Five messenger RNAs (mRNAs) for production of the viral proteins are transcribed, and a full-length, positively stranded RNA is produced as the template for the viral progeny. The envelope forms from host cisternal membranes into which the G and M proteins are inserted. In natural infections the virus accumulates in cytoplasmic cisterns from which it is released either by membrane fusion or dissolution of the cell.

VIRUS SUSCEPTIBILITY:

Rabies virus does not with stand heat. The viruses will be having the half-life of about less than one minute at 56^0C when compared to several hours at 37^0C. At 4°C, there is little loss after 2 weeks. The lipid coat is disrupted by detergents or a 1% soap solution. Other virucidal agents include

iodine solutions (1:10000 available iodine), 45% ethanol and 1% benzalkonium chloride, but phenol is not so effective.

Susceptibility of rabies virus

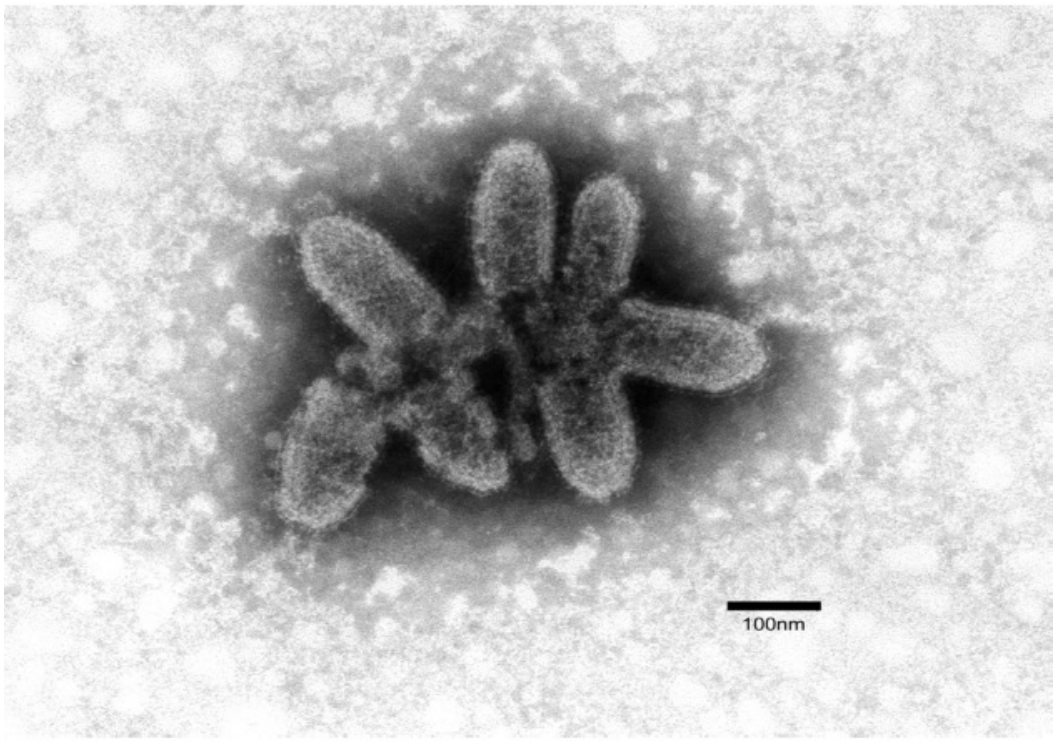
Inactivation of rabies virus

- At 60°C within 35 seconds (sensitive to pasteurisation and boiling)
- At pH < 4 or > 10
- By action of oxidizing agents, most organic solvents, surface acting agents, quaternary ammonium compounds, proteolytic enzymes, ultraviolet rays and X-rays
- Soaps and detergents
- Alcohol

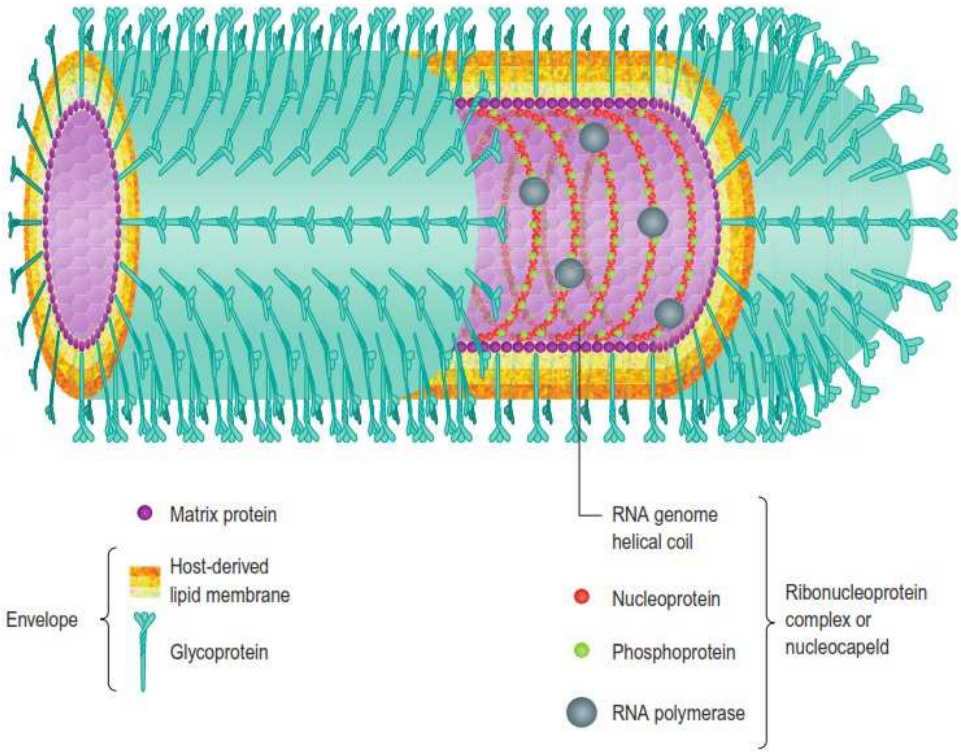
Preservation of rabies virus

- By freeze drying
- At ultra low temperatures (≤ - 20°C)
- Glycerine

ELECTRON MICROSCOPE VIEW OF CLUMPS OF RABIES VIRUS:



COMPONENTS OF RABIES VIRUS:



RABIES VIRAL GENOME AND ITS FUNCTION:

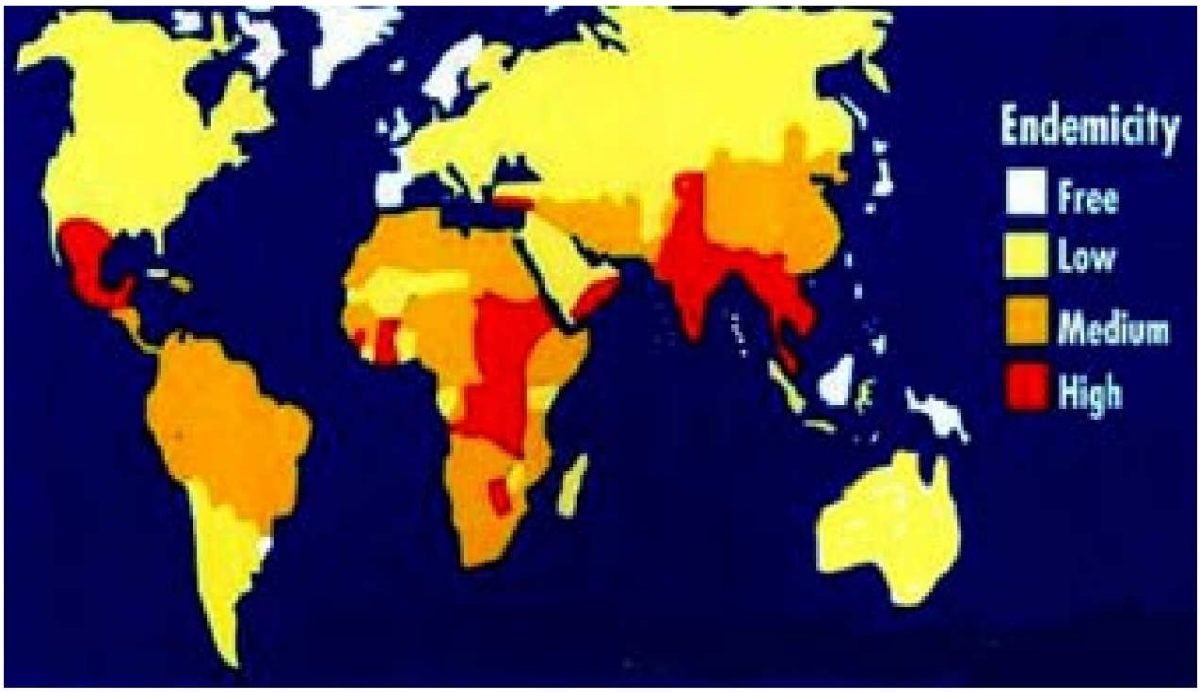
Gene	*Synonyms*	*Size (kDa)*	*Function*
N (nucleocapsid)	—	50	—
NS (nonstructural)	M_1, P	40	Originally thought to encode a nonstructural protein but now known to produce a structural protein that is phosphorylated by kinases in the host cell and that joins with L.
M (matrix)	M_2	26	Responsible for the assembly and budding of bullet-shaped particles, in concert with the G protein.
G (glycoprotein)	—	65	Attachment to host cell receptors.
L (large)	—	160-190	RNA-dependent RNA polymerase; required for transcription of the negatively stranded viral RNA; appears to form a complex with NS.

EPIDEMOLOGY:

Rabies is a viral zoonos is causing almost uniformly fatal encephalitis in humans and other mammals. Human rabies is one of the most under reported diseases worldwide. Rabies is enzootic worldwide. The terrestrial mammals are free of rabies in Western Europe and Australia but rabies-related lyssa viruses are found to be present in bats.

Important reservoirs are domestic dogs, jackals, mongoose, bats, skunks, raccoons, coyote, and wolves. In India dogs, wolves, jackals forms

the important link. Rabies related deaths are high in India, Bangladesh, Pakistan, and Thailand.



Global status of rabies

Susceptibility of Various Animal Species to Rabies			
Very High	*High*	*Moderate*	*Low*
Wolves	Hamsters	Dogs	Opossums
Foxes	Skunks	Primates	
Coyotes	Raccoons		
Kangaroo rats	Domestic cats		
Cotton rats	Rabbits		
Jackals	Bats		
Voles	Cattle		

Animals transmitting rabies in India

Frequent	Occasionally		Not reported
Dogs & cats	Monkeys Cows & buffaloes Mongoose Foxes, Wolves & jackals Sheep & goats	Bears Pigs Donkeys Horses Camels	Bats * Rodents * Birds Squirrel

Note: All exposures in wild are considered as Category III exposures.

* Bite by bats or rodents do not ordinarily necessitate rabies vaccination in India. However, bites by bats or rodents in unusual circumstances may be considered for vaccination in consultation with an expert in the field of rabies.

Mode of transmission

Common	Rare
• Bites from infected animals • Licks on broken skin and mucous membrane • Scratches	• Inhalation • Organ transplantation • Ingestion

TRANSMISSSION OF INFECTION:

ANIMAL CONTACT:

Humans get infected by virus laden saliva, inoculated during bite of rabid animal into a wound or on to a mucous membrane may result in infection. Intact skin serves as a barrier for viral entry.

HUMAN CONTACT:

There are some anecdotal reports of transmission of infection via contact with human saliva, kissing, biting, sexual intercourse, breast feeding, and eating infected meat but they are largely unproven and viraemia in blood has not been detected

In contrast to events in neurons, virus replication in acinar cells of the salivary glands produces large amounts of extracellular virus. Although there is no evidence of viraemia, rabies virus is shed in human lachrymal and respiratory tract secretions and possibly in urine and in milk.

Transmission has occurred through *grafting of infected corneas*. Six virologically proven cases followed transplants from donors with unsuspected rabies. In Texas and in Germany, mysterious encephalitis killed a total of seven *organ transplant* recipients. The documented evidence regarding rabies viral transmission via transplantation exist for

1 .Liver

2. Kidney

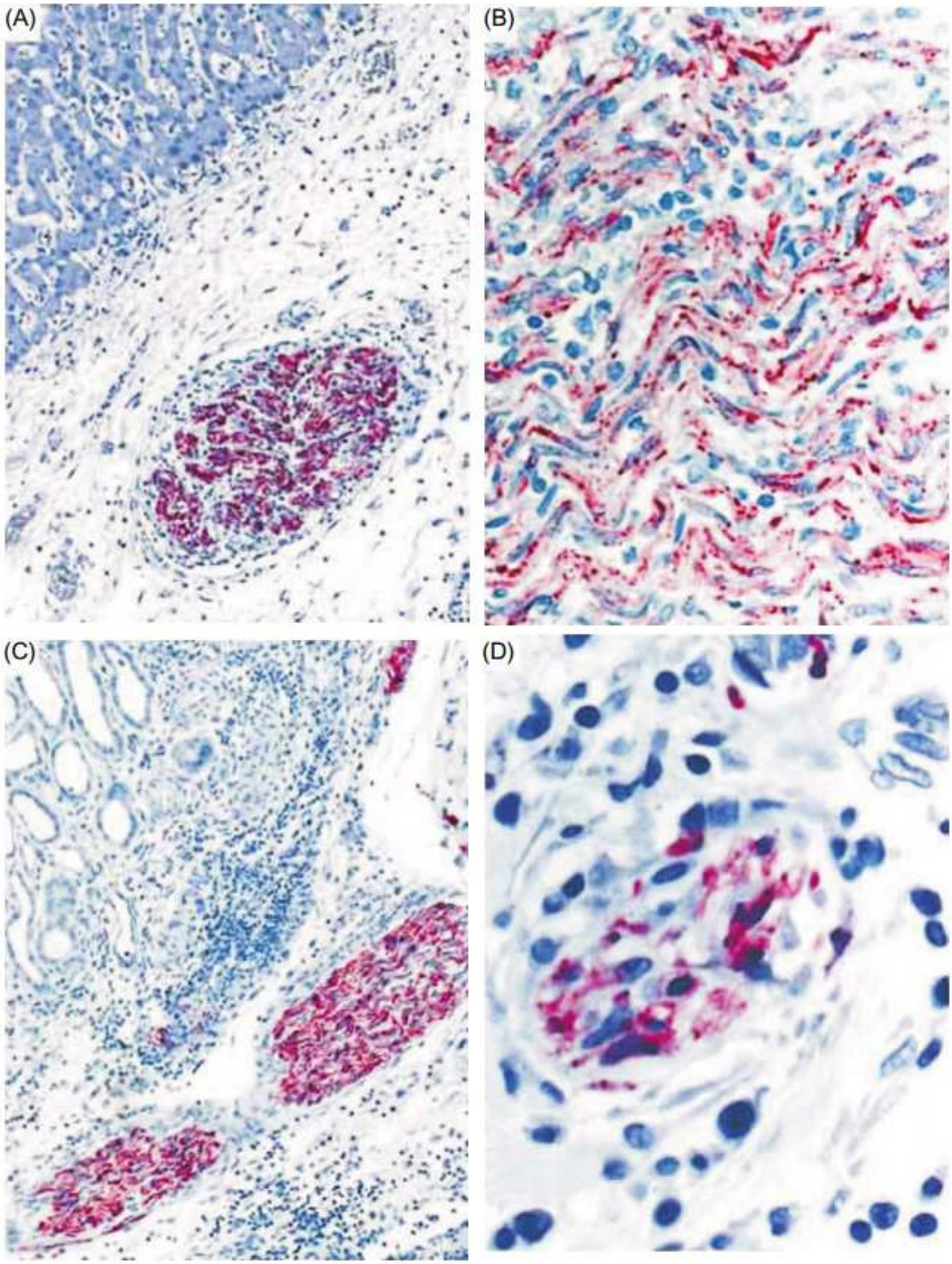
3.Pancreas

4. Iliac artery grafts

The above said grafts have been harvested from two young donors died of undiagnosed neurological disease. Later through history and background check revealed a previous bat bite in USA and contact with a dog in India. Two cornea transplant recipients were given rabies post-exposure prophylaxis and remain healthy.

Transplacental infection occurs in animals, and a Turkish woman and her 2-day-old infant died of virologically confirmed rabies. This is exceptional. Many mothers with rabies have been delivered of healthy babies.

Two rabies infections resulted from inhaled aerosols of ‘fixed’ Virus in laboratory accidents. Two more people were possibly infected by inhalation of aerosolized virus in bat-infested caves, but direct bat contact was not excluded. Butchering dogs or cats is another source of rabies, but consuming the cooked rabid meat did not cause infection.



Immunohistochemical staining for rabies virus antigen (red) in peripheral nerves of the liver (A and B), kidney (C), and arterial graft (D) transplants.

PATHOGENESIS:

After virus inoculation into the wound or contact with mucous membrane it replicates in the muscle cells by doing so it infects the muscle spindles, via the muscle spindle it then infects the nerve that innervates the muscle spindle then it moves centripetally in the axons of the infected nerve via fast axon transport. The virus is first seen in the dorsal root ganglion before spinal cord which confirms the transport of rabies virus via sensory nerves.

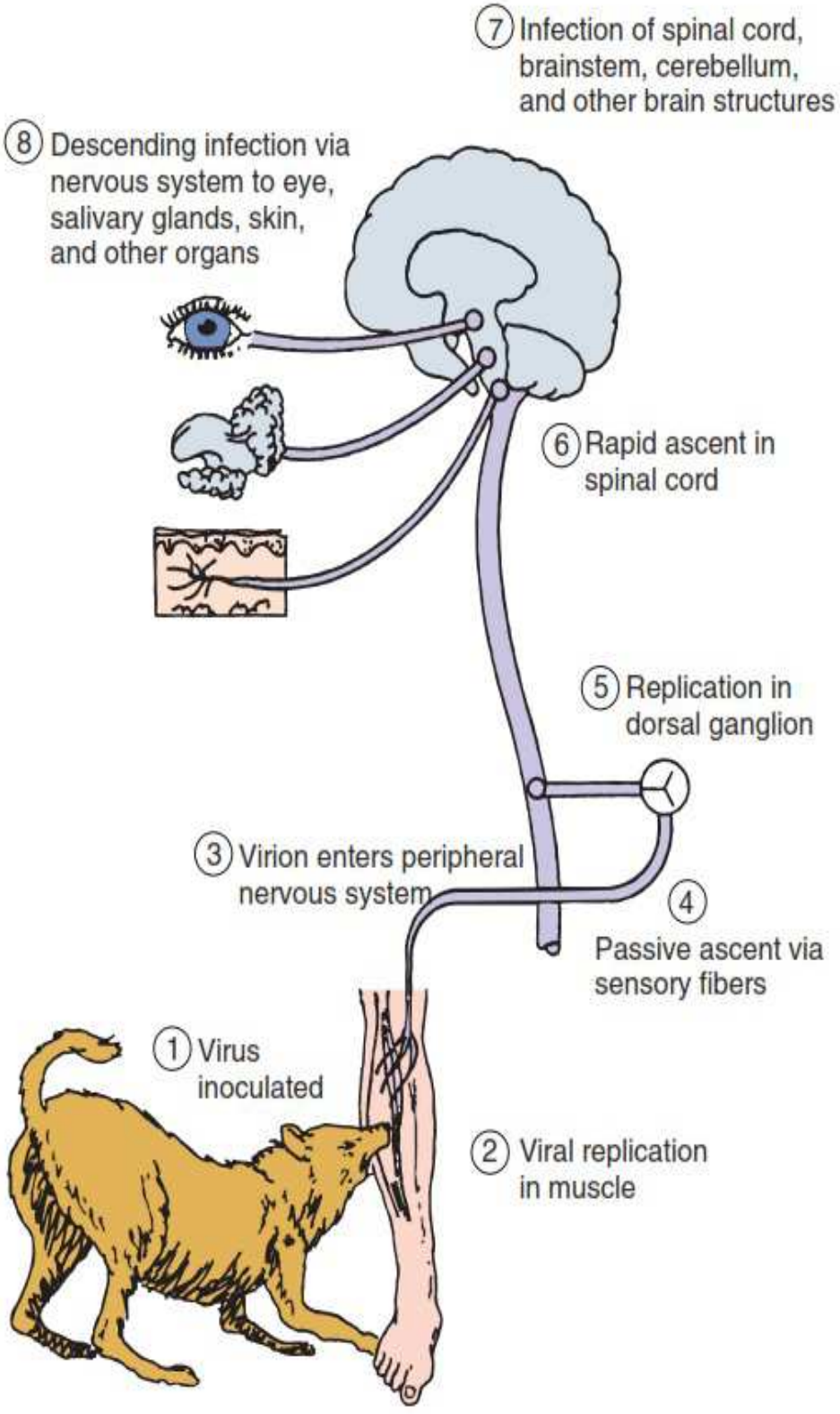
Some studies suggest the neuromuscular junction plays a major role in neuronal invasion because there is a partial sequence homology exists between snake neurotoxins and rabies viral glycoprotein that plays an important role in adhesion. There is also evidence that by blocking acetylcholine receptors viral attachment can be inhibited. However, rabies virus can enter neurons that do not express acetylcholine receptors but with less efficacy therefore other receptors must exists for viral attachment

Natural course of infection consists of local viral replication before central nervous system infection, this is perhaps to increase the inoculum before nervous system infection. Thus timely administration of rabies immunoglobulin and active immunization can prevent the infection in to

nervous system and thus the disease. Once the virus gains the entry into nervous system the current therapeutic measures available won’t be much helpful.

After reaching spinal cord it spreads to entire central nervous system via synaptic connectivity. From the central nervous system it spreads to the entire body via peripheral nerves.

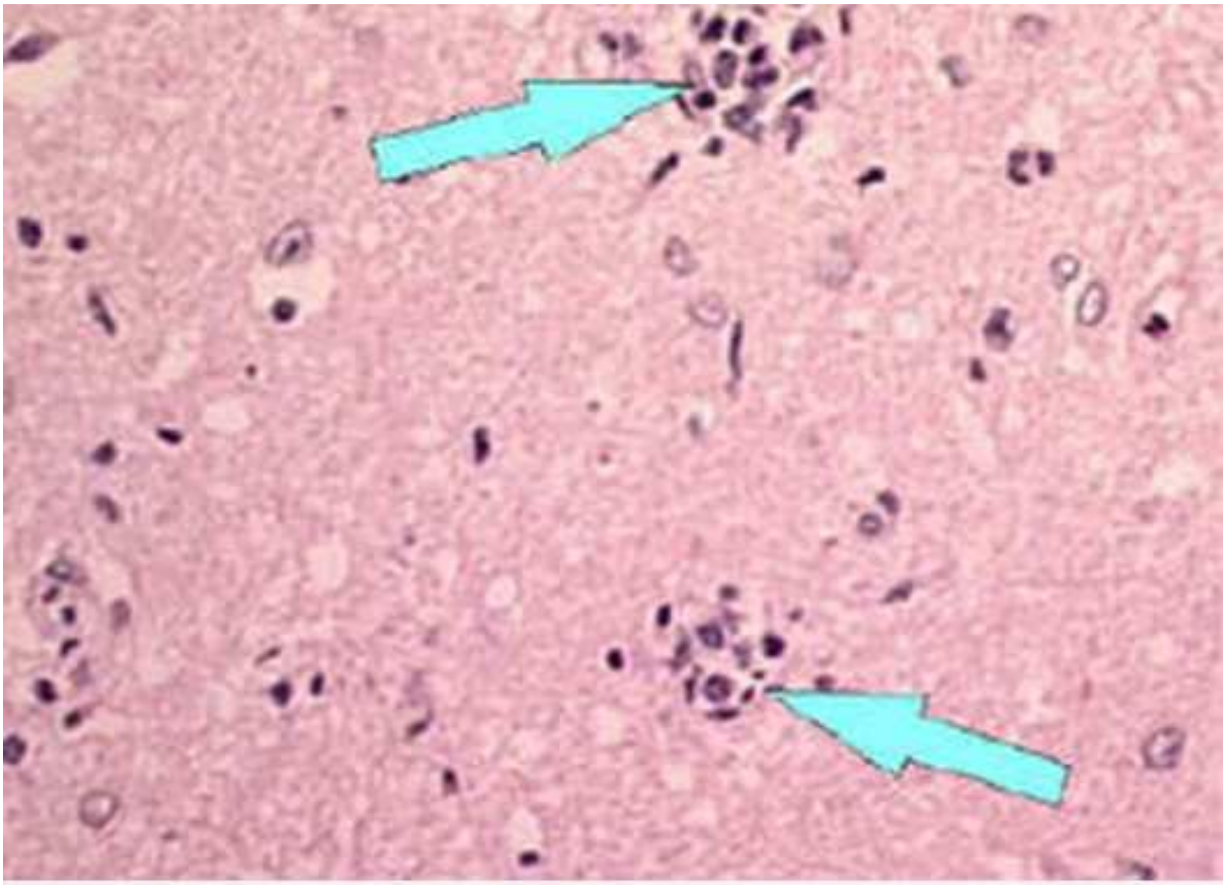
Mechanism of rabies viral damage to central nervous system remains obscure because the neuronal necrosis is very minimal or either absent. The virus may interfere with neurotransmission. There is also evidence suggestion excitotoxic mechanism by increasing nitric oxide levels up to 30 fold increase. The rabies virus infection also capable of inducing apoptosis in in T lymphocytes this may form the basis of failure of immune system to clear the virus.



Pathogenesis of rabies virus infection. Numbered steps describe the sequence of events.

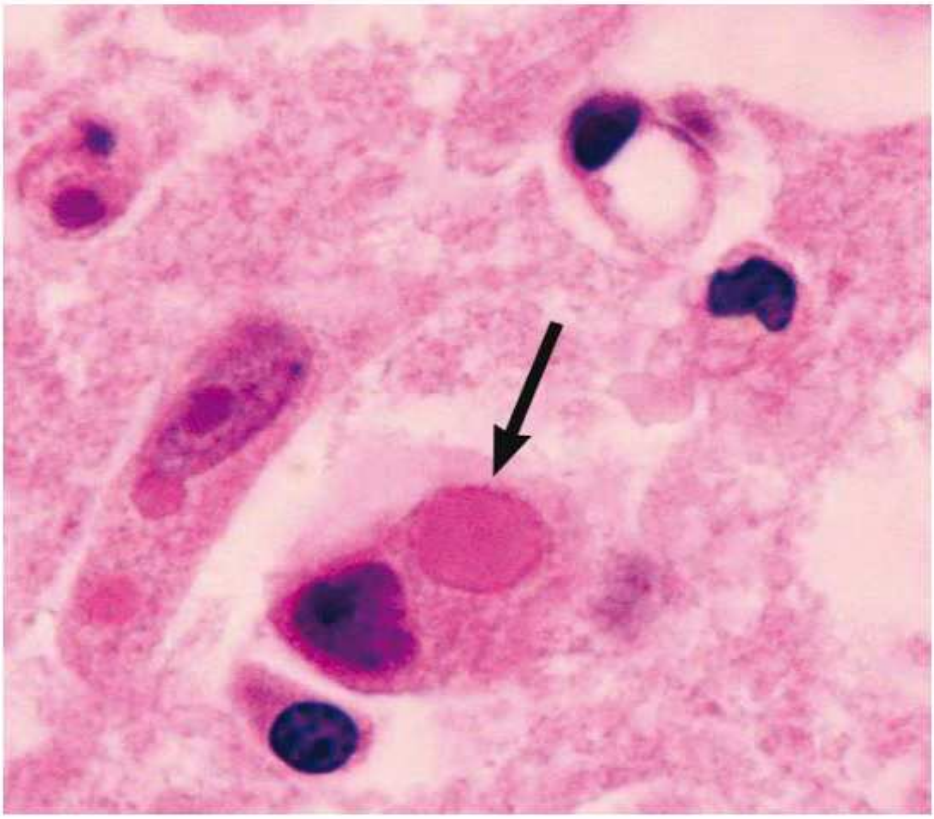
PATHOLOGY:

Gross brain specimen of rabies encephalitis mainly consist of cerebral congestion and few petechial hemorrhages. Microscopically lymphocytic perivenous infiltrate can be observed. There may be focal areas of demyelination which may mimic Guillian Barre syndrome like polyneuropathy. There may be perineural infiltration called *Babes nodules* can be present.



Babes Nodules

The pathognomic finding of *Negri bodies* is found only in 75% of cases. They are found in hippocampus, purkinje cells of cerebellum and in medulla .the involvement of hippocampus explains about the aggressive behavior of the infected persons and animal. These above said findings are present mainly in furious rabies



Negri body *(arrow)*. Original magnification, ×400.

Negri bodies are oval eosinophilic cytoplasmic inclusion bodies ranging from 1– 7 micrometers. They mainly consist of viral nucleocapsids. Lyssa bodies are structurally similar to Negri bodies. They are also isolated from heart. Involvement of heart resembles myocarditis in hypercatecholamines states like pheochromocytoma, sub arachnoid

hemorrhage and tetanus. Adelchi Negri first thought that Negri bodies are protozoan organisms and named it as *Neurocytes hydrophobiae.*

Paralytic rabies mainly affects spinal cord with necrosis and severe inflammation. Brain stem is involved to lesser extent in paralytic or dumb rabies.

IMMUNE RESPONSE:

The natural immune response to rabies virus infection is insufficient to prevent the development of disease which once developed is invariably fatal. Rabies viral infection can produce immunosuppression by inducing apoptosis of T lymphocytes. Antibodies levels that can be measured can develop late in the course of the disease by the time it is too late. Those who develop cellular immunity to develop furious rabies and die faster than those who don't have such a response these are the patients who develop paralytic rabies. Immunosuppression by rabies can also be explained by the production of interleukin 1 in the central nervous system. There is one theory that rabies virus persist inside macrophages and emerge later to cause the disease. This may be explain the very long incubation seen in some cases.

CLINICAL MANIFESTATIONS OF HUMAN RABIES:

The variables affecting the development of disease following exposure depends upon

1. Viral inoculum – the chances of are viral inoculum is more when the rabid animal bites the exposed rather than the areas covered by means of clothes. Because clothes may remove the saliva at the time of bite.
2. Probability increases with multiple bite wound rather than single, but not always.
3. Location of bite site [proximity to brain] – bites on faces are more likely to cause disease than those on the extremities.

Incubation period: varies from few days to 90 days

There is evidence that rabies encephalitis can present after an incubation period of 25 years

CLASSIFICATION:

Based on the clinical manifestations rabies is classified into

1. Furious or encephalitic rabies
2. Dumb or paralytic rabies

In the prodromal phase it could not be differentiated from other viral illness based on the symptoms only. The furious form presents with hydrophobia, aerophobia, delirium and agitation. The paralytic form can present as an ascending paralysis. In either the case course usually last for 2 to 14 days before the coma supervenes. Once the coma settles in death usually occurs in 1 to 2 weeks. Cardiac arrhythmias mainly supraventricular can occur which may be due to brainstem dysfunction or can be due to myocarditis. Autonomic disturbances also seen like piloerection, marked salivation, anisocoria and rarely priapism and spontaneous ejaculation. Weakness is the main presentation in paralytic type which can more in the extremity where the viruses are introduced.

Myoedema is present during the prodromal phase and it persists throughout the disease.

Clinical features in humans

Prodormal phase	Discomfort or pain at the site of bite Numbness and tingling in limb Elevated temperature Malaise Headache Sore throat Priapism
Excitation phase	Restlessness, tremors Pharyngeal and laryngeal spasms Fear of water (**hydrophobia**) Terror and excitement Respiratory arrythmias Cardiac arrythmias Hypertension Convulsions on exposure to light air sound **Intense thirst and dehydration** Change in tone of voice Death
Paralytic phase	Restlessnes, convulsions Flaccid and limp muscles Unconsciousness Death

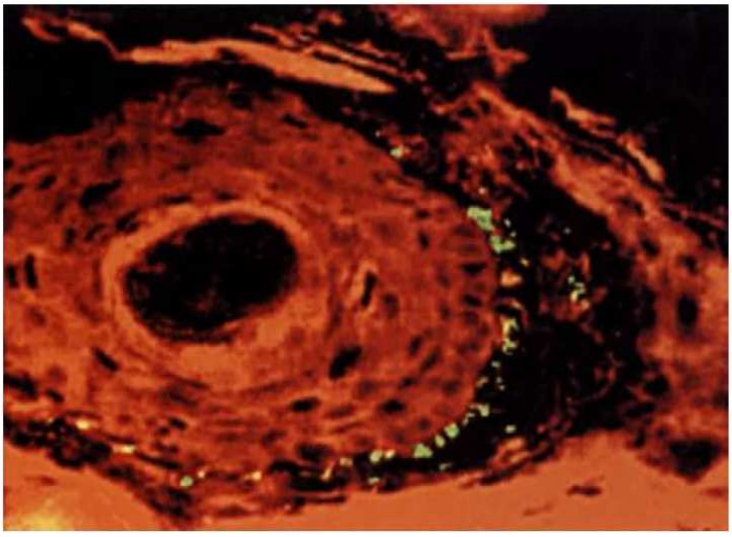
Durations of Different Stages of Rabies

Stage	*Duration (% of Cases)*	*Associated Findings*
Incubation period	<30 d (25%) 30-90 d (50%) 90 d to 1 y (20%) >1 y (5%)	None.
Prodrome and early symptoms	2-10 d	Paresthesias or pain at the wound site; fever; malaise; anorexia; nausea and vomiting.
Acute neurologic disease; Furious rabies (80% of cases)	2-7 d	Hallucinations; bizarre behavior; anxiety; agitation; biting; hydrophobia; autonomic dysfunction; syndrome of inappropriate antidiuretic hormone (SIADH).
Paralytic rabies (20% of cases)	2-7 d	Ascending flaccid paralysis.
Coma, death	0-14 d	—

DIAGNOSIS:

The diagnosis is straight forward in those presenting with hydrophobia after bite by a rabid animal. But rabies should be suspected in every individual presenting with progressive encephalitis regardless of the history of dog bite or exposure. Ante mortem diagnosis of rabies viral infection requires several specimens like saliva, serum, urine, and CSF, and also requires multiple testing modalities because none of the test is highly sensitive and the viral shedding can be intermittent.

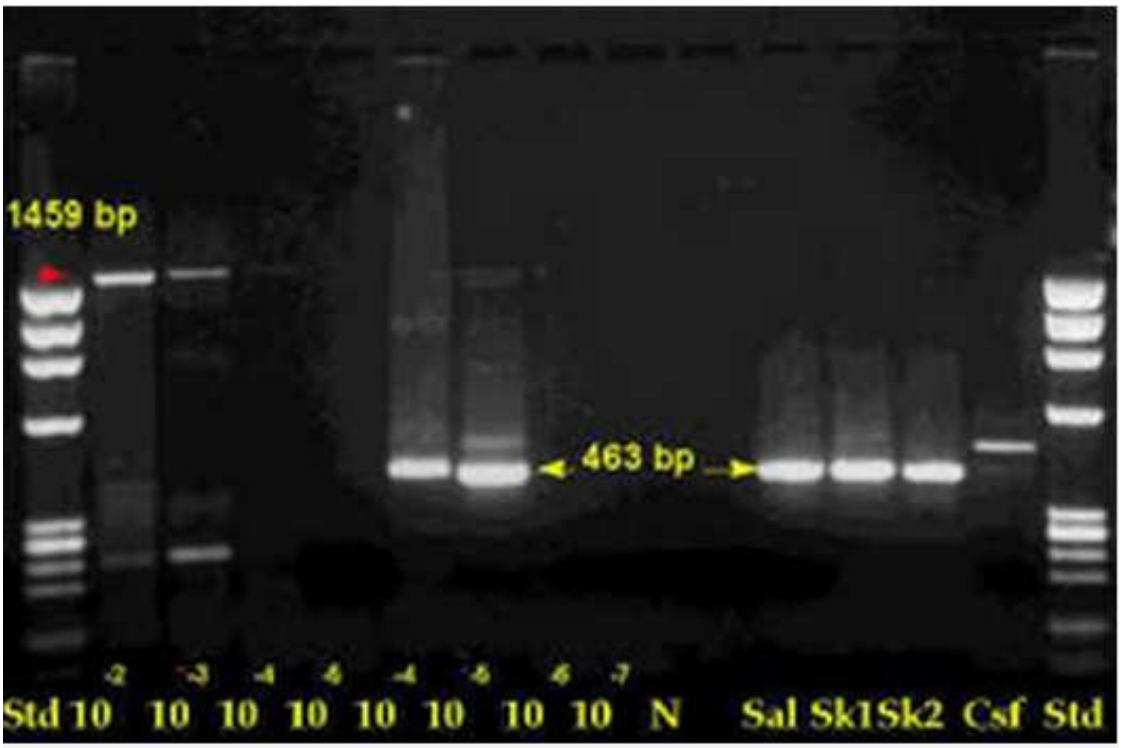
Direct fluorescent antibody staining of biopsy material mainly from the nape of the neck above the hairline because the viral tends to localize in those hair follicles because it is richly innervated by nerve fibers. During the first week about 50% of samples reveal rabies the percentage stars increasing after that.



Human hair follicle from nuchal skin biopsy. Nerve fibers surrounding the follicle are stained by specific fluorescence indicating the presence of rabies virus antigen (direct fluorescence antibody method on a frozen section; ×250 magnification).

Reverse transcriptase – polymerase chain reaction [RT-PCR]:

It can be performed on CSF, saliva, and on tissues. It allows geographic and host species origin of particular rabies virus. It can also be performed on decomposed brain tissues. The sensitivity of saliva sample can be increased to 100% if three samples can be obtained and tested.

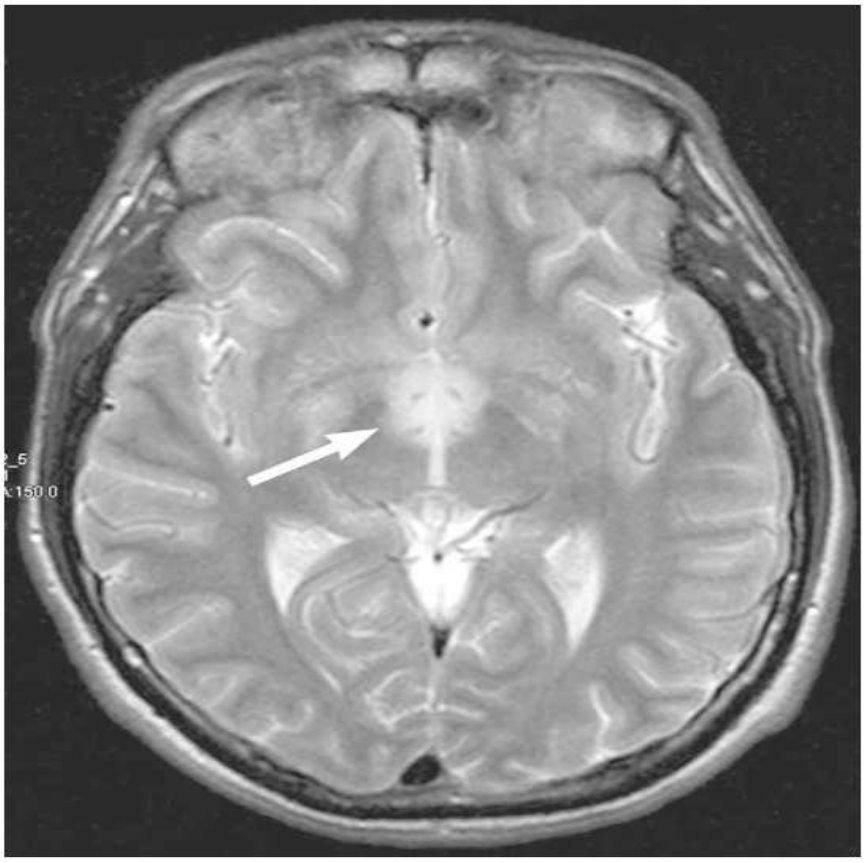


PCR test results for rabies virus. The arrows indicate positions of positive bands.

The rapid fluorescent focus inhibition test [RFFIT] is a test for virus neutralizing antibodies,50% develop these antibodies in detectable though not protective levels by day 8 and 100% by day 15. Any amount of antibodies levels in CSF is diagnostic even though the patient has received the post exposure prophylaxis.

If no active or passive immunization has been given, the presence of antibodies to rabies virus in serum is diagnostic. But if the person has been given vaccination second serum sample has to be obtained few days to demonstrate the increase in titers for diagnosis. However irrespective of vaccination status of the individual antibodies in CSF is diagnostic of infection.

Imaging studies are not specific for rabies. MRI brain can show increased T2 weighted signal intensities in hippocampus, hypothalamus and brainstem regions



T2-weighted MR image showing increased signal in diencephalon *(arrow)*.

Laboratory Diagnostic Methods

Sample		Method
Full thickness skin* punch biopsy	Antigen detection	IFA test on frozen section† RT-PCR
Saliva or throat swab* Tears CSF	Virus isolation and antigen detection	Tissue culture Mouse inoculation test RT-PCR
Serum	Antibody test ‡	Presence of antibody is diagnostic in unvaccinated patients If previously vaccinated, save sample for comparison later
CSF	Antibody test ‡	Test in parallel with serum
Brain post-mortem: needle necropsy§ or autopsy sample brain stem and cerebellum	Virus isolation and antigen detection	Tissue culture mouse inoculation test IFA test on impression smear RT-PCR

*Most useful samples for antigen detection, repeat daily until a diagnosis is confirmed.
†Rabies antigen seen in nerve twiglets around the base of hair follicles by immunofluorescence (IFA) test.
‡Immunofluorescent antibody test is rapid and sensitive. Neutralizing antibody test takes ≥2 days.
§Necropsies are taken with a long biopsy needle via the medial canthus of the eye; through the superior orbital fissure; via the nose through the ethmoid bone; or through the foramen magnum or open fontanelles in children.

Interpretation of ante-mortem diagnostic tests

- Difficult – require battery of tests
- No single test has been positive in every case
- Distribution of rabies antigen in nuchal biopsy or corneal impression may be extremely irregular
- False positive results have been obtained in fluorescent examination of corneal epithelium
- Antibody response to infection on occasions may be absent
- It is necessary to test repeated samples
- Samples should be tested using all currently available tests
- Ante-mortem diagnosis should be attempted only by experienced laboratories
- A negative diagnosis does not rule out rabies

ADVANTAGES AND LIMITATIONS OF INVESTIGATIONS

FOR RABIES:

Test	Advantages	Limitations
Seller's Staining for inclusion bodies (Negri bodies)	• Simple • Rapid (1 hour) • Easy to perform • No special equipment required	• Positive in 50 – 70% of cases
Fluorescent Antibody Test (FAT) for antigen detection	• Specific (near 100%) • Sensitive (near 100%) • Relatively Rapid (1 day) • Easy to perform	• Expensive • Good Fluorescent microscope • Good quality conjugate • Trained manpower
Mouse Inoculation Test (MIT) for virus isolation	• Can detect very small quantity of virus • Confirmatory	• Takes long time (21 days) • More of academic value • Use of laboratory animals (mice). Thus laboratories should have a well maintained animal house. • Ethical issues involved in use of laboratory animals
Rapid Tissue Culture Infection Test (RTCIT) for virus isolation	• Rapid (4 days) • Sensitive	• Special cell culture laboratory • Expensive • Trained manpower

PREVENTION:

With no effective treatment options available once the disease is confirmed, the main stay of focus is on prevention by means of pre as well as post exposure prophylaxis

DIFFERENT VACCINATION PREPARATIONS:

Louis pastuer pioneered the work on rabies vaccination. In 1885 Pauster successfully immunized the first patient Joseph Meister with his rabies vaccine. In 1911 Sir David Semple prepared an inactivated carbolized vaccine that was derived from infected animal nervous tissues which became Semple vaccine. This was used in many countries for many years. Due to frequent neuroparalytic complications this vaccine was not used now a days.

Now only cell cultured vaccines and purified duck embryo vaccine are used. The neurotropic vaccine in India was stopped since December 2004.“Anti-rabies vaccines are produced as one single intramuscular dose with potency of > 2.5IU per IM dose for post exposure and pre-exposure prophylaxis. It is absolutely essential that every batch of cell culture vaccine or purified duck embryo vaccine have minimum potency of 2.5IU per intramuscular dose”.

The vaccine can be given either intra dermal or intra muscular route. The vaccine are available in both freeze dried lyophilized as well as liquid adsorbed forms. Shifting from one brand or type of vaccines are not encouraged, but in case of unavoidable situations this adjustments are not forbidden.

CURRENTLY AVAILABLE ANTIRABIES VACCINES IN INDIA:

	Brand	Product	Pharmaceutical
1.	Abhayrab	Purified Vero cell Rabies Vaccines (PVRV)	Human Biologicals Institute, Hyderabad
2.	Indirab	Chromatographically purified (PVRV)	Bharat Biotech International Ltd, Hyderabad
3.	PVRV*	Purified Vero cell Rabies Vaccine (PVRV)	Pasteur Institute of India, Coonoor, Tamilnadu
4.	Rabipur	Purified Chick Embryo Cell Vaccine (PCECV)	Novartis Vaccines, Mumbai
5	Rabivax	Human Diploid Cell Culture Vaccine (HDCV) (Liquid)	Serum Institute of India, Pune
6	Vaxirab	Purified Duck Embryo Vaccine (PDEV)	Zydus Health Care ltd., Ahmedabad
7	Vaxirab-N	Purified Chick Embryo Cell Vaccine (PCECV)	Zydus Health Care Ltd, Ahmedabad
8.	Verorab	Purified Vero cell Rabies Vaccines (PVRV)	Sanofi Pasteur/ Zuventus Health Care, Mumbai
* Limited production, since July 2001.			

Anti rabies vaccines available in India

Name of the vaccine	Fixed virus strain	Substrate	Available
1. Neural tissue vaccine BPL inactivated sheep brain vaccine (Semple type)	PV – 11	Sheep brain	Production stopped since December 2004
2. Cell Culture vaccines i) Human Diploid Cell Vaccine (HDCV) ii) Purified Chick Embryo Cell Vaccine (PCEC) iii) Purified Vero Cell Rabies Vaccine (PVRV)	Pitman Moore (PM) LEP-Flury Pitman Moore (PM)	MRC-5 Primary SPF chick embryo cells Vero Cells	Produced locally in private. Sector Produced locally in private sector Imported + produced locally in public & private sector
3. Purified Duck Embryo Vaccine	Pitman Moore (PM)	Duck Embryo	Produced locally in private Sector

VACCINES APPROVED FOR ID USE IN OUR COUNTRY:

Only the vaccines approved by DCGI for intradermal route should be used for intra dermal route. Irrespective of reconstituted volume each dose at single intra dermal site should consist of 0.1ml of vaccine. Once reconstituted vaccine should be used within 8 hours. During that period the reconstituted vaccine should be stored at a temperature of 2-8ºC. Intra dermal route for anti-rabies vaccination was by world health organization since the year 1992.

VACCINES APPROVED FOR IM ROUTE IN OUR COUNTRY:

1. Human diploid cell vaccine-HDCV, liquid [adsorbed] 1ml
2. Purified chick embryo cell vaccine - PCECV 1ml
3. Purified Vero cell culture vaccine – PVCV o.5ml and 1 ml
4. Purified duck embryo vaccine –PDEV 1 ml Of the above four, the first three vaccines are cell cultured vaccines

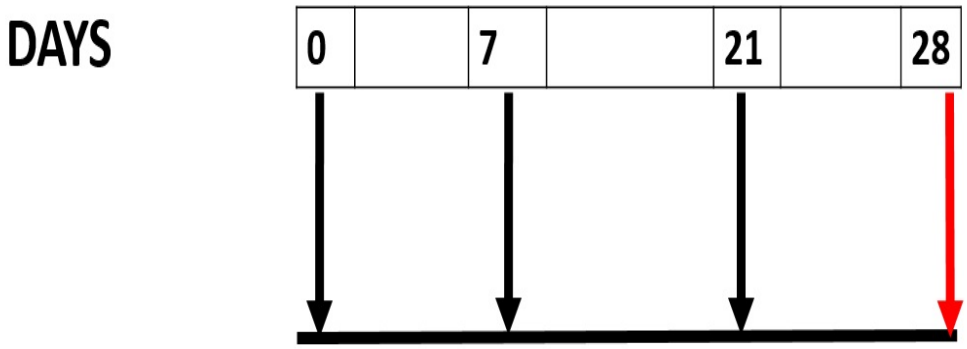
PRE-EXPOSURE PROPHYLAXIS:

It may be offered to those high risk individuals like

1. Laboratory staff handling the virus and infected material
2. Clinicians and health care workers handling human rabies cases
3. Veterinarians
4. Animal handlers and animal catchers
5. Quarantine officers
6. Travelers from rabies free areas to rabies endemic areas

The Indian academy of pediatrics recommended pre exposure prophylaxis to children on voluntary basis.

SCHEDULE:



EACH ARROW MARK INDICATES TWO SITE INTRADERMAL VACCINATION OF O.1ml DOSE. THE NUMBERS INDICATE THE DAYS. '0' DAY BEING THE DAY OF IMMUNISATION NOT THE DAY OF DOG BITE. THE VACCINATION CAN BE GIVEN ON 0,7,21 DAYS OR 0,7,28 DAYS. LONGER THE LAST DAY OF IMMUNISATION LONGER THE IMMUNITY WILL LAST. THE VACCINES CAN ALSO GIVEN AS IM ROUTE BUT THE DOSE IS O.5ml OR 1.0ml

POST EXPOSURE PROPHYLAXIS:

It has to be approached in a stepwise manner. Three steps has to be given equal importance and should be followed in a stepwise manner as far as possible.

1. Categorization of animal bite wounds and proper wound management.

2. Passive immunization with rabies immunoglobulin either with human or equine origin.

3. Active immunization with anti-rabies vaccination.

It should be emphasized that post exposure prophylaxis should be started as soon as possible after the exposure. However it should not be denied to those presenting late.

It is one of the few conditions that both active and passive immunizations are given on the same time but at different sites.

Post exposure is the main stay in preventing rabies since no effective treatment options available. When administered properly it is nearly always 100% effective. Local multiplication of rabies virus before entering into the nervous system gives us the time for post exposure prophylaxis.

World health organization has classified wounds into three categories regarding post exposure prophylaxis

WHO WOUND CLASSIFICATION REGARDING POST EXPOSURE PROPHYLAXIS:

WHO Classification of Wound			
Recommended post-exposure prophylaxis category	**Type of contact with a suspect or confirmed rabid domestic or wild[a] animal, or animal unavailable for testing**	**Type of exposure**	**Recommended post-exposure prophylaxis**
I	Touching or feeding of animals Licks on intact skin	None	None, if reliable case history is available
II	Nibbling of uncovered skin Minor scratches or abrasions without bleeding	Minor	Administer vaccine immediately[b] Stop treatment if animal remains healthy throughout an observation period of 10 days[c] or if animal is proven to be negative for rabies by a reliable laboratory using appropriate diagnostic techniques
III	Single or multiple transdermal bites or scratches, licks on broken skin, contamination of mucous membrane with saliva (i.e. licks), exposures to bats[d]	Severe	Administer rabies immunoglobulin and vaccine immediately. Stop treatment if animal remains healthy throughout an observation period of 10 days or if animal is found to be negative for rabies by a reliable laboratory using appropriate diagnostic techniques

[a] Exposure to rodents, rabbits and hares seldom, if ever, requires specific anti-rabies post-exposure prophylaxis.

[b] If an apparently healthy dog or cat in or from a low-risk area is placed under observation, the situation may warrant delaying initiation of treatment.

[c] This observation period applies only to dogs and cats. Except in the case of threatened or endangered species, other domestic and wild animals suspected as rabid should be humanely killed and their tissues examined for the presence of rabies antigen using appropriate laboratory techniques.

[d] Post-exposure prophylaxis should be considered when contact between a human and a bat has occurred unless the exposed person can rule out a bite or scratch, or exposure to a mucous membrane.

WOUND MANAGEMENT:

It mainly consists of these steps

1. Physical treatment
2. Chemical treatment
3. Tetanus prophylaxis

Wound(s) Management

<table>
<tr><th colspan="3">Do's</th></tr>
<tr><td>Physical</td><td>Wash with running water</td><td>Mechanical removal of virus from the wound(s)</td></tr>
<tr><td>Chemical</td><td>Wash the wound(s) with soap and water
Apply disinfectant
   </td><td>Inactivation of the virus</td></tr>
<tr><td>Biological</td><td>Infiltrate immunoglobulin into the depth and around the wound(s) in Category III exposures
  </td><td>Neutralization of the virus</td></tr>
<tr><th colspan="3">Don'ts</th></tr>
<tr><td colspan="3">• Touch the wound(s) with bare hand
• Apply irritants like soil, chilies, oil, lime, herbs, chalk, betel leaves, etc.</td></tr>
</table>

PHYSICAL MANAGEMENT:

Wound toilet should be done by prompt and gentle washing under running water. It should be done for 15 to 20 minutes. It should be kept in mind that wounds should not be handled with bare hands all the time. Sterile gloves should be used. The wound washing should not be denied to those persons presenting late if the wound is unhealthy. The benefit for fresh wounds is maximum when compared with the old and unhealthy wounds.

CHEMICAL MANAGEMENT:

Followed by wound toileting with running water, wounds should be treated with soap and detergents because virus is inactivated by the above agents. Then antiseptics can be applied. Antiseptics like iodine and alcohol can be used. The application of the irritants to the wounds is unnecessary and it can be damaging.

SUTURING:

Suturing of wounds should be avoided as far as possible. But if surgically unavoidable after adequate wound washing and local infiltration with passive immunization loose sutures can be applied after delaying for several hours. The delay in suturing and lose sutures helps in diffusion of antibodies into the tissues.

PASSIVE IMMUNISATION:

The anti-rabies immunoglobulin both human as well as the equine can be used for passive immunization. They provide the readymade antibodies that can bind with the viruses in the muscle tissue before it gains entry into the nervous system. They tie over the period required for the production of antibodies by means of active immunization.

WOUND CAUTERISATION:

It is contraindicated because it leads to bad scar formation as well as it does not contain any additional benefits.

TETANUS PROPHYLAXIS:

If required tetanus prophylaxis should be provided to prevent the sepsis and a course of antibiotics can be prescribed if needed.

ANTIRABIES IMMUNOGLOBULIN:

Human antirabies immunoglobulin: 20IU/Kg dose

Equine antirabies immunoglobulin: 40IU/Kg dose

Human antirabies immunoglobulin is preferred over equine whenever available because of homologous origin and free of side effects encounter in when using heterologous origin like equine immunoglobulin. Because of the longer half-life only half the dose required for equine immunoglobulin is

used. The main limiting factor for its use is the cost. It was in 1890 *BABES* demonstrated the usefulness of anti-rabies immunoglobulin in experimental animals. And then till 1960 equine immunoglobulin was used but it was not purified and this leads to many adverse effects. Now highly purified and enzyme digested ERIG are available leading to very much less adverse effects.

The use of human serum for making rabies immunoglobulin was introduced by *HOSTY* in 1959. By 1971 *CABASSO* made standardization in the production of human antirabies immunoglobulin and determined the optimal dose required for passive immunization.

EQUINE ANTIRABIES IMMNUGLOBULIN:

ERIG is produced by hyperimmunisation. They contain highly purified Fab2' fragments. There is possibility of adverse effects because of its heterologous origin but it is virtually zero by means of highly purified techniques. However literature evidences support that skin testing prior to administration does not predict anaphylactic reactions. There is also need to give equine immunoglobulin if human immunoglobulin is not available. Therefore routine skin testing is not recommended. The treating physician should be prepared to manage anaphylaxis even though rare it can occur at any stage of administration.

INDICATION OF PASSIVE IMMUNISATION:

1. All category III exposures
2. Bites by wild animals
3. Additionally in Category II exposures of immunocompromised individuals
    - If an individual consumed the milk of a the rabid animal without boiling full dose of immunoglobulin has to given via IM route followed by full course of rabies vaccination
    - Following sexual exposure with a human rabies patient full dose of immunoglobulin has to be given via IM route followed by full course of rabies vaccination.

There is no contraindication for immunoglobulin use in pregnant and lactating females. The dosage of immunoglobulin should not exceed the recommended dose calculated. It should be infiltrated immediately as possible soon after the cleaning of wounds. It can be given along with rabies vaccination but at a different site. However if only rabies vaccination alone was started initially, the immunoglobulin can be given up to 7 days after starting the first dose. After that period it will interfere with the production of antibody stimulated by means of active immunization. Hence it is not advisable to give immunoglobulin 7 days after starting active immunization.

By this time antibody production by active immunization will start and able to protect the individual.

ADMINISTRATION OF IMMUNOGLOBULIN:

Before administration the immunoglobulin must be brought to room temperature. It is important to infiltrate all immunoglobulin to al wounds to neutralize the virus locally. Systemic administration via IM route is of very little value. The total dose of immunoglobulin calculated should be infiltrated as anatomically feasible, into and around all wounds.

During injection of immunoglobulin the piston of syringe should be pushed slowly while withdrawing the needle to ensure that the entire thickness of the wound is infiltrated. It is preferable to use separate injections for different wound sites. Multiple injections into the same wound should be avoided as far as possible. Care should be taken to in avoiding compartment syndrome while injecting immunoglobulin in fingertips and toes. After infiltrating all areas if small volume of immunoglobulin is left it can be given systemically via IM route at a distant site. Gluteal route should be avoided.

Rabies immunoglobulin can be diluted with sterile normal saline if the requirement for infiltration is high.

- If the exposure involves the eyes, the immunoglobulin can be used as eye drops. For this purpose it has to diluted with normal saline in the ratio of 1:1
- If the wound is sutured because of severe blood loss or in any unavoidable situations rabies immunoglobulin can still be given into the sutured wounds without disturbing the sutures. In those cases it can be seen to ooze from the wound site after injection. If too much oozing is seen it has to be compensated
- In cases of already infected wounds immunoglobulin can be administered after thorough cleaning.
- It should never be given via intra venous route.

Erythema and serum sickness may develop following rabies immunoglobulin administration in 1 to 2% of patients after six days. In that case antihistamines and non-steroidal anti-inflammatory drugs are sufficient. They are observed in equine immunoglobulin not during human immunoglobulin administration.

POST EXPOSURE PROPHYLAXIS [ACTIVE IMMUNISATION]:

The two types of vaccines now available in India are

1. Tissue cultured vaccine
2. Purified duck embryo vaccine

It can be via either intramuscular or intradermal methods. There are many regimens for vaccination which evolved over time. The number of doses required becoming less and less. The number of days required for vaccination also been reduced over the time. All of these are achieved mainly because of the development of more potent vaccines and development made in understanding the immunological basis of active immunization for rabies.

INTRA MUSCULAR REGIMEN:

The approved regimen for intramuscular route are

1. Essen regimen
2. Zagreb schedule

ESSEN REGIMEN:

There are two recommendations regarding this regimen

1. World health organization recommendation
2. Center for disease control – Advisory Committee on Immunization Practices.

WHO RECOMMENDATION: [1-1-1-1-1]

WHO recommends five dose intramuscular regimen. It consists of five doses of recommended amount of approved vaccines via intramuscular administration. The days the vaccines should be given are 0, 3, 7, 14, and 28.

The sixth dose on day 90 should be considered optional for individuals who are immunologically deficient, those who are in extremes of age and who are all on immunosuppressive therapies. The day '0' indicates the date of first dose of vaccination and not the day of dog bite. The vaccines should be given in deltoid region. Gluteal region is to be avoided because of higher fat layer and chances of vaccines being deposited in fat is higher when compared to deltoid region. The vaccine must be deposited in muscle for inducing immunogenicity. For children who are less than 2 years of age the vaccines are to be given in the anterolateral aspect of thigh.

CDC – ACIP RECOMMENDATIONS: [1-1-1-1]

The CDC - ACIP on 2010 came with the recommendation that the fifth dose on day 28 is not needed based on the retrospective analysis of the data. The regimen now being followed in United States of America is four dose regimen via intramuscular route on days 0, 3, 7, and 14. The doses on 28th and 90th days are given for those on extremes of ages, those who are on immunosuppressive therapies and who are immunodeficient. The recommendation regarding immunoglobulin use by WHO is not changed.

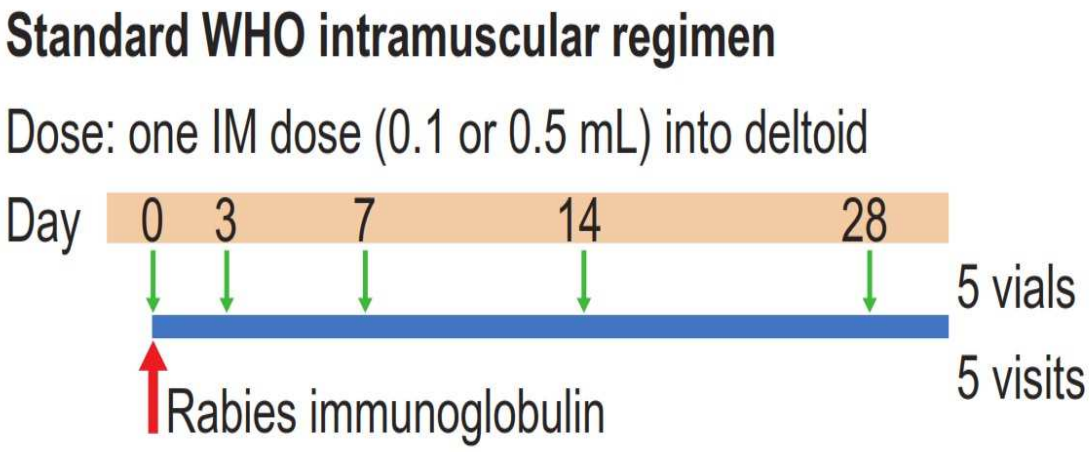
ZAGREB REGIMEN: [2-1-1]

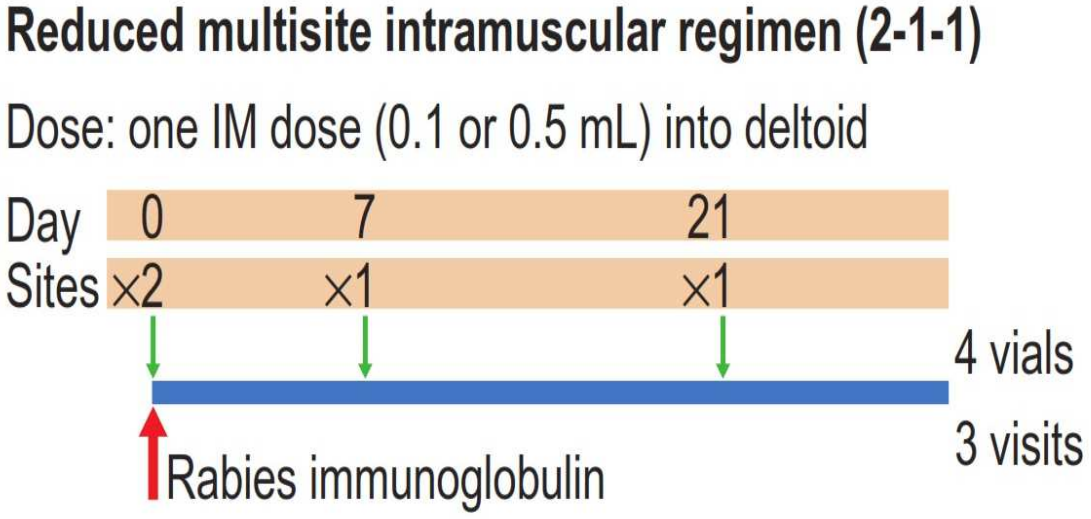
Abbreviated multisite intramuscular regimen. It consists of two doses of vaccines on day '0', one dose on each deltoid on day '0'. Followed by one

dose of vaccine on day 7 and day 21. By means of this schedule number of visits and doses of vaccines are also reduced. The WHO also approved this regimen.

SPECIAL SITUATIONS: [2-1-1-1-1]

In conditions where immunoglobulin is not available there should be more emphasis on proper wound toileting, this should be followed by Essen regimen of tissue culture vaccination with double dose of vaccine on day '0' at 2 different sites intramuscularly. The remaining doses on days 3, 7, 14 and 28. The doubling the dose of vaccine on first day is done for faster induction of immune system in producing the antibodies. But it should be greatly emphasized that doubling the dose on the first day is not a replacement to immunoglobulin.





INTRADERMAL REGIMEN:

There are three regimens for intradermal vaccination schedule

1. Updated Thai Red Cross schedule
2. Oxford – eight site intradermal regimen
3. Four site intradermal regimen

UPDATED THAI RED CROSS REGIMEN: [2-2-2-0-2]

One dose of vaccine, that is o.1ml/0.2 ml of vaccine is given at two different lymphatic drainage sites usually in the right and left upper arm on days 0, 3, 7, and 28**.**The day '0' corresponds to the day of vaccination not the day of dog bite.

The vaccines approved for this regimen in India is

1. Purified vero cell vaccine
2. Purified chick embryo cell vaccine

8 SITE INTRADERMAL REGIMEN–OXFORD REGIMEN [8-0-4-0-1-1]

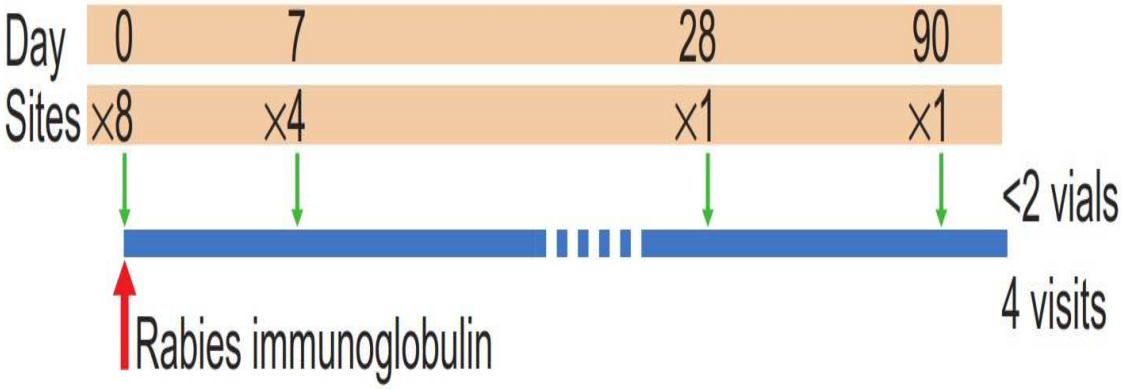
It consist of one dose of 0.1ml of vaccine administered at eight different lymphatic drainage system – two upper arms, two thighs, either side on lower quadrant of abdomen, both suprascapular region on day '0'. Then on day '7' four o.1ml on arms and thigh region. Followed by one injection on 0.1 ml in arm on days 28 and 90. The day '0' corresponds to the day of vaccination and the day of vaccination.

The vaccines approved for this regimen are

1. Human diploid cell vaccine
2. Purified chick embryo cell vaccine

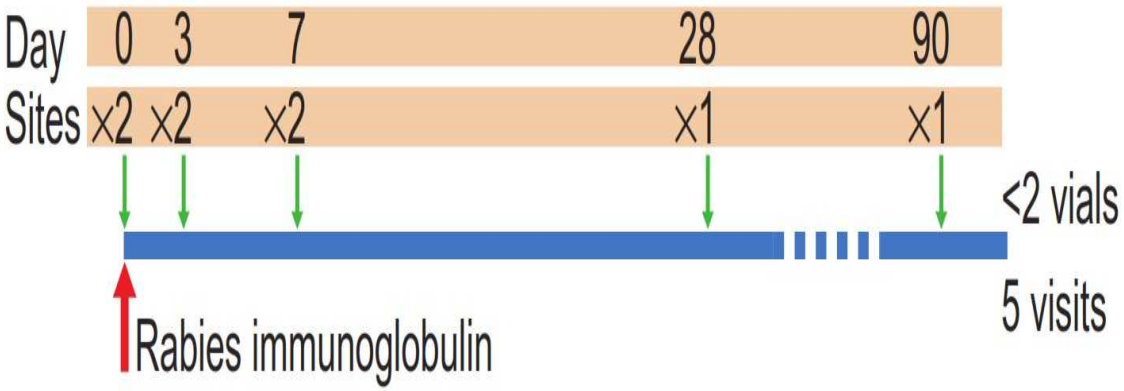
8-site intradermal regimen (8-0-4-0-1-1)

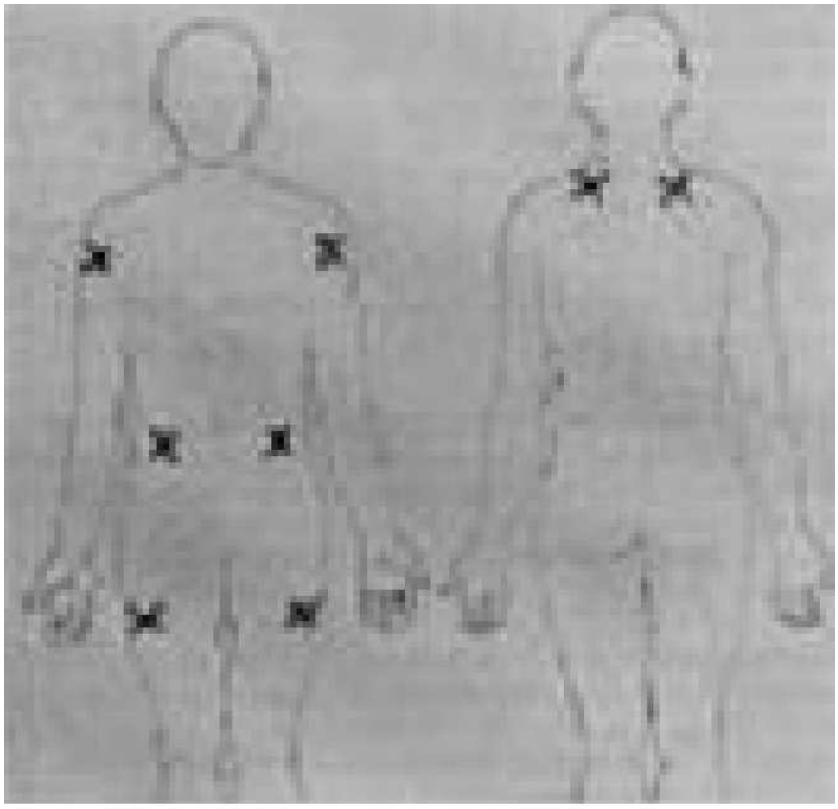
Dose: 0.1 mL ID per site



2-site intradermal regimen (2-2-2-0-1-1)

Dose: one ID dose = one fifth of IM dose (0.1 or 0.2 mL), ID per site





: Sites for ID inoculation in 8-site ID regimen

FOUR SITE INTRADERMAL REGIMEN: [4-0-2-0-1]:

The vaccines approved for this use are

1. purified vero cell vaccine
2. purified chick embryo cell vaccine

It consist of four doses of 01.ml/0.2ml of vaccine on day ‘0’. Each dose to be injected intradermally at four different lymphatic drainage sites. On day 7 it consist of two doses at two different sites. On day 28 it consist of

one dose of vaccine. Here also the day '0' corresponds to day of vaccination not the day of dog bite.

NOTE: in cases of highly potent vaccine like purified vero cell culture vaccine each dose of vaccine is 0.1ml but in cases of other vaccines the dose of each vaccine is 0.2 ml

VACCINE STORAGE RECONSTITUTION AND USAGE:

Anti-rabies vaccines are available in both liquid form and in lyophilized form. Though lyophilized form is stable to mild temperature changes it has to be stored at 2-8^{o} C. Even though freezing does not damages the lyophilized powder there is a chance that ampoule containing diluent may break. Liquid vaccines should never be allowed to be frozen.

The lyophilized vaccines should be reconstituted with the diluent provided immediately prior to the usage. Once reconstituted the vaccines should be used within 6-8 hours. Otherwise it has to be discarded. During those periods it has to be stored in a refrigerator.

Switching from one brand to another brand during the course of vaccination is not recommended. "Currently anti rabies vaccines are produced as one intra muscular dose with potency of greater or equal to 2.5IU per IM dose for both pre as well as post exposure prophylaxis". It is absolutely essential that every batch of cell cultured or purified duck

embryo vaccine should have a potency of above said levels for its use in general population.

MECHANISM OF ACTIVATION OF IMMUNE SYSTEM:

When vaccines are given via intramuscular route the antigen gets deposited in the muscle. After that it is absorbed by the blood vessels and then presented to the antigen presenting cells which then activates the immune system. The amount of vaccine required for the activation of immune system is more when compared to the intradermal dose. It thus have a large economic concerns.

When vaccines are given via intradermal route at multiple lymphatic drainage sites its gets deposited in the dermal layer of the skin. The antigen is directly carried by the antigen presenting cells via the lymphatics to the regional lymph nodes and regional reticulo endothelial system there by eliciting prompt and protective antibody response. When given via intradermal route the amount of vaccine required to elicit the immune response is only a fraction of the level required when given via intramuscular route. It is actually one fifth to one tenth of dose of intramuscular route.

When using the intra dermal route the countries able to cover large population with the limited quantities available. This is especially useful in cases of economically constrained countries.

Based on the above said facts who recommends the vaccination against rabies via intradermal route from 1992 onwards. India has also adopted the WHO recommendation.

RESPONSE TO IMMUNISATION:

Early experiment studies revealed that the inactivated viruses used in the active immunization leads to the stimulation of the production of cytotoxic T – cells. There is also contradicting evidence to this observation, the depletion of cytotoxic T – cells in experimental mice had no effect against rabies virus protection nor in the survival rate of vaccinated animals. This evidences points out that cytotoxic T – cells alone not responsible for protection against rabies. There is also evidences pointing to the involvement of B – cells as wells as CD4+ helper cells via MHC II mechanisms. These leads to the activation of lymphocytes and antibody producing plasmocytes. Thus rabies virus neutralizing antibodies are produced. Then they migrate to the neural parenchyma. CD4+ T lymphocytes activation plays an important role in the protection against rabies virus via vaccination. Thus both humoral and cell mediated immunity plays a vital role in the protection against rabies.

The role of cell mediated and humoral immunity is studied in human models also by comparing immune response pattern with those having combined B cell and T cell immunodeficiency. The healthy individuals produced significant increase in the rabies virus immunoglobulin of IgM type starting one week after vaccination. Then one week later there is also significant increase in the IgG type also specifically IgG1 and IgG3. The above antibodies are measured using ELISA technique. Then after the booster dose the levels of IgG levels increases more than it does following the primary vaccination. This response is measured one week after the booster dose. Overall IgG1 plays an important role in the rabies immunity both in the primary as well as booster vaccination. In the same study involving the B and Tcell immunodeficient individuals they found number of abnormalities in the immune response.

Following inoculation the virus either multiply in the muscle before entering into the peripheral nervous system or it can directly enter the nervous system. The clear-cut pathological mechanism is yet to understood. Once the rabies virus gains entry into the nervous system the prognosis is always nearly fatal. Thus highlighting the importance of rabies immunoglobulin in post exposure prophylaxis. There is evidence also that even after rabies virus entry into the nervous system neutralization is still possible which is supported by the fact that active immunization given after

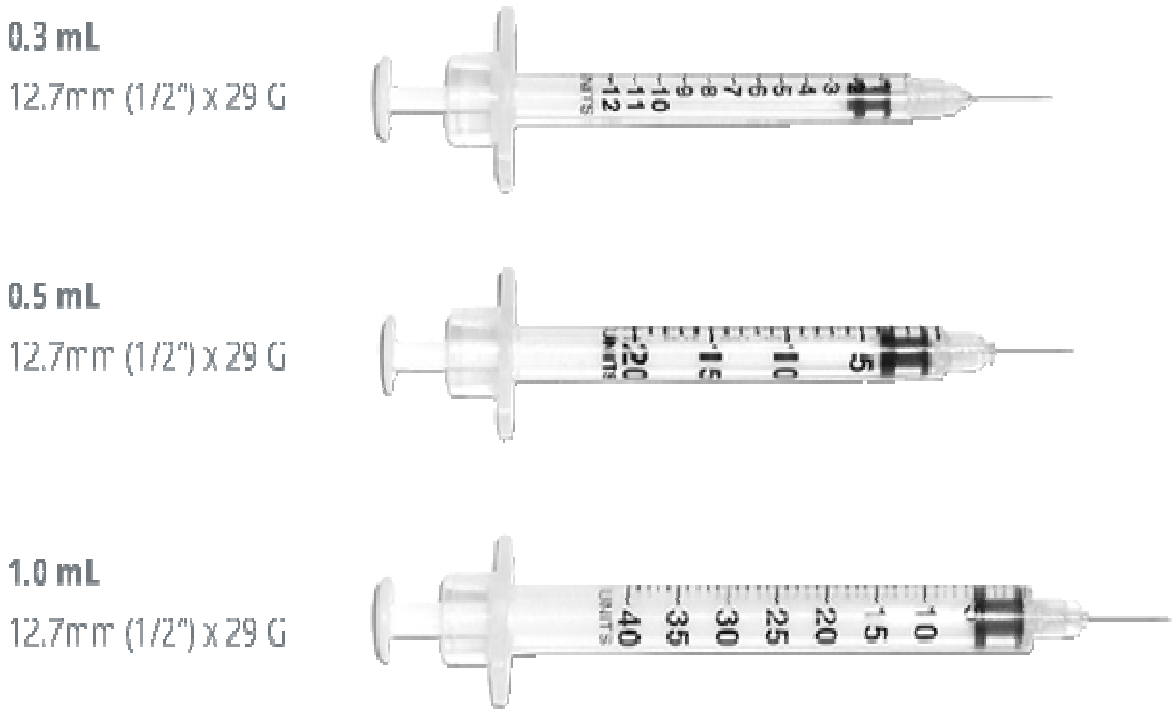
several days to months can still able to protect the individual. This forms the basis that rabies immunization should not be denied for those who are presenting late. Thus it can be taken that rabies virus antibodies can occasionally clear virus from the central nervous system.

After the active immunization against rabies the antibodies are produced against the various components of the viral proteins specifically G and N components. The experimental evidences indicate that antibodies directed against N component alone do not confer protection even though they are the main components raised after the immunization. The role of non-neutralizing antibodies against the rabies virus is not clearly understood till now. There is no specific protective rabies virus neutralizing antibody levels that can be named as protective. Most of us consider that an antibody level of 0.5 IU/ml is protective. The WHO has given this level only as a proof of adequate immune response not as the protective level.

Those who are taking steroids, anti-malarials especially chloroquine, immune compromised should receive active immunization via intramuscular route and not via intradermal route, as well those who are on extremes of age. They might also require addition doses of vaccines. These are peoples where testing of antibody levels will be very much useful.

TECHNIQUE OF INTRADERMAL VACCINATION:

As said above that the individual should not be immune compromised or should not be on any immunosuppressive drugs, chloroquine when planned to receive the vaccination via in tradermal rote.



The vaccines approved for intradermal route only should be used. If the vaccine used is lyophilized vaccine it has to reconstituted with the diluent provided by the manufacturer in the prescribed amount using 2ml syringe with the 24G needle. The reconstituted volume is 1ml mostly.

The vaccine dose to be given at one site is o.1ml in case of vero cell vaccine and 0.2ml in cases of less potent other cell culture vaccines and purified duck embryo vaccine. This amount is constant irrespective of the reconstituted volume used for reconstitution.

For intradermal vaccination it preferable to use either 20 units or 40 units insulin syringe with 29G needle. Because of less painful experience as well as entering the dermal layer will be easier when compared with the standard intramuscular syringes. It should be the self-mounted needle to decrease the vaccine wastage when compared to the detachable needles.

1 ml 40 UNITS INSULIN SYRINGE: 4 units equals 0.1ml

0.5 ml 20 UNITS INSULIN SYRINGE: 4 units equals 0.1 ml

Depending upon the vaccines used 4 or 8 units of vaccine will be taken in the insulin syringe after sterile aseptic precautions. Air should be let out before the injection to avoid dead space in the injection. Then using the BCG injection technique the needle is inserted parallel to the skin after stretching the skin surface. The bevel should face upwards at the time of injection. If the needle is correctly placed in the dermal layer there will be resistance to the injection. A bleb should be raised after successful injection into the dermal layer. Surface of the skin will show the puckering and feel like peel of the orange “peau d’ orange”.

If the vaccine is injected deep into the subcutaneous layer it will not raise the bleb. Then the needle should be withdrawn and injected at a different site once more.

The sites used for intradermal injection are

1. over the deltoid region
2. suprascapular region
3. anterior aspect of thigh region
4. lower quadrants of abdomen

- After injection do not rub or apply anything to the injection site.
- Advice the patient about the need for the completion of vaccination for the effective immune response.

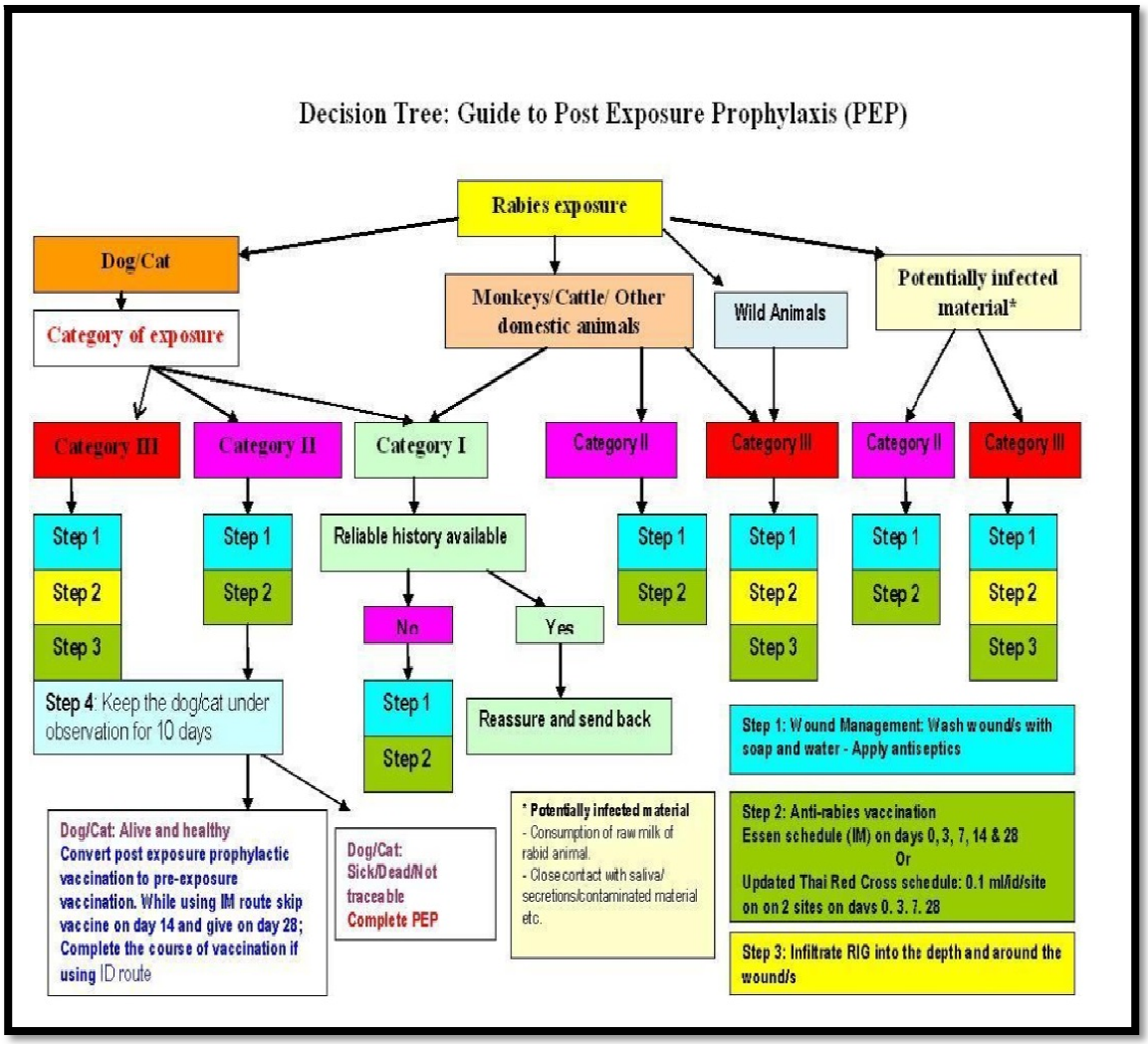
ADVERSE REACTIONS:

Mild adverse reactions like itching and erythema, fever can occur in small number of patients. They are self-limiting and life threatening. Symptomatic management like analgesics and anti-histamines are usually sufficient. Steroids should be avoided as they interfere with the immune response

NOTE: switching over from one brand to another brand of vaccine during the course of vaccination is not recommended.

Switching from one route of administration to another during the course of vaccination like from intradermal to intramuscular or vice versa is not recommended.

GUIDE TO POST EXPOSURE PROPHYLAXIS:



POST EXPOSURE PROPHYLAXIS OF PREVIOUSLY VACCINATED INDIVIDUALS:

For those individuals who are vaccinated previously by means either pre exposure or post exposure prophylaxis on re-exposure should receive one of the following methods recommended below

1. IM booster regimen 0, 3 day regimen
2. ID booster regimen 0, 3 day regimen
3. Single day 4 site ID booster regimen

IM BOOSTER REGIMEN:

The two booster via intramuscular route on day 0 and day three produces effective anamnestic response regarding antibody production. This regimen was approved by WHO since 1992.

ID REGIMENS:

Similar to intramuscular route vaccination days the vaccines are given on day 0, and 3. The dose being 0.1ml in case of purified vero cell culture vaccine and 0.2ml in case of other vaccines. They are given at only one site. Even though the seroconversion rate is slower when compared to intramuscular route this regimen was approved by WHO since 1992.

There is an alternative single day regimen when using intradermal route. It consists of four doses of vaccines at four ID sites on the same day. This regime is also approved by WHO

NOTE: Rabies immunoglobulin is not needed for those individuals who are previously vaccinated by tissue cultured or purified duck embryo cell vaccine.

Because the rabies immunoglobulin will suppress the anamnestic response. But if neuro trophic vaccines were used during pre-exposure or post exposure prophylaxis that individual should receive rabies immunoglobulin during re-exposure like the vaccine naïve individual. When the individual has no memory regarding the vaccine type used during previous vaccination or when there is no records available of prior vaccination they require rabies immunoglobulin during re-exposure.

Booster doses are recommended every 6 months for those individuals who are at very high risk like those working in rabies virus laboratories. Every 6 months they has to undergo rabies virus neutralizing antibody testing and if the levels are below the protective levels booster doses are required.

For those individuals having frequent contacts with animals booster doses are required every two years once. For others no booster doses are routinely required.

Comparison of selected rabies vaccine regimens.

Vaccine Regimen & Route	Days of Injection Superscript: Number of sites injected						Visits to Clinic	Total Vaccine Used
Pre-exposure								
IM	0		7			28	3	3
ID 0.1 ml[a]	0		7			28	3	<1–3[b]
Post-exposure (+RIG day 0)								
IM 5 dose	0	3	7	14		28	5	5
IM 2-1-1	0^2		7		21		3	4
IM 4 dose (CDC)	0	3	7	14			4	4
2-site ID	0^2	3^2	7^2			28^2	4	<2–4[b]
4-site ID[c]	0^4		7^2			28	3	<2–3[b]
Post-exposure if previous vaccine course								
IM	0	3					2	2
ID[d]	0^4						1	1 (or <1)[d]

[a] ID doses are all 0.1 ml per site of injection.
[b] Maximum used if ampoules not shared.
[c] ID doses are 0.1 ml/site for PVRV vaccine (0.5 ml/ampoule) or the equivalent dose 0.2 ml/site for PCECV vaccine (1.0 ml/ampoule).
[d] 0.1 ml/site, whole ampoule used for 0.5 ml vaccines. 1 ml ampoules share between two, alternatively use whole dose to avoid wastage.

NOTE:

When persons are vaccinated via intradermal route the amount of neutralizing antibody attained is higher as well as the immunological memory persist longer when compared to persons received the vaccine via intramuscular route. But when booster doses are needed the vaccine given via intramuscular route induces rapid anamnestic response when compared to intradermal route. Because of the concerns in the technique of the intradermal injection and multiple punctures needed intramuscular route is adopted by the developed nations. When giving intradermal injection instead of depositing in the dermal layer if the vaccine is deposited in the muscle layer it will not lead to effective stimulation of immune system because intradermal route uses

only a fraction of dose of intramuscular route. But there are reports despite of above concerns intradermal vaccination against rabies is being effectively implanted in many countries including India.

RABIES TRAETMENT:

Till now there is no effective treatment options available once the diagnosis is made. There is protocol called "**Milwaukee Protocol** or **Wisconsin protocol"** which claimed to be the best treatment option available, but it fails to produce any beneficial effects in clinical experimental studies. Till now there are seven human rabies documented to be survived but based on the personal communications two of them found to be dead. So at present there are only five human rabies survivors. Of those five survivors there is only one personal with complete recovery, others are having mild to severe neurological sequelae. In all those documented recoveries post exposure vaccination was given to all of them except one. One of those survivors is a laboratory worker who exposed to live rabies virus has already underwent pre exposure vaccination.

All the human rabies case survivors are diagnosed on the basis of rabies virus neutralizing antibodies in the CSF. No rabies virus was isolated from their skin biopsy or corneal smear except one individual. From that individual rabies viral RNA was isolated from the skin biopsy.

There also two reports of rabies viral antibodies present in the CSF without the presence of rabies viral neutralizing antibodies in their CSF. They did not any clinical symptoms suggestive of rabies. There was no contact history elicited from them. The exact etiology and pathogenesis remain unclear.

Cases of Human Rabies with Recovery

Location	Year	Age of Patient	Transmission	Immunization	Outcome	Reference
USA	1970	6	Bat bite	Duck embryo vaccine	Complete recovery	(Hattwick et al., 1972)
Argentina	1972	45	Dog bites	Suckling mouse brain vaccine	Mild sequelae	(Porras et al., 1976)
USA	1977	32	Laboratory (vaccine strain)	Pre-exposure vaccination	Severe sequelae	(Tillotson et al., 1977a, 1977b)
Mexico	1992	9	Dog bites	Post-exposure vaccination (combination)	Severe sequelae[a]	(Alvarez et al., 1994)
India	2000	6	Dog bites	Post-exposure vaccination (combination)	Severe sequelae[b]	(Madhusudana et al., 2002)
United States	2004	15	Bat bite	None	Mild to moderate sequelae	(Willoughby et al., 2005; Hu et al., 2007)
Brazil	2008	15	Vampire bat bite	Post-exposure vaccination	Severe sequelae	(Ministerio da Saude in Brazil, 2008)

[a]*Patient died less than 4 years after developing rabies with marked neurological sequelae (L. Alvarez, personal communication).*
[b]*Patient died about 2 years after developing rabies with marked neurological sequelae (S. Mahusudana, personal communication).*

NOTE:

- In the above mentioned human rabies cases the second case in 1972**,** the vaccine used in the post exposure prophylaxis is neurotropic vaccine. This patient also showed prominent cerebellar sighs even though the rabies virus can infect the cerebellum human rabies patients will not show clinical manifestations of cerebellum. It should be also noted that this patient attained almost complete recovery with very minimal neurological sequelae. This also sparkles the thinking that this could be a case of neuroparalytic complications of neurotropic vaccination rather than a case of human rabies.
- In the above mentioned cases the third case in a laboratory personal who has already completed pre exposure prophylaxis exposed while working with live rabies virus via aerosol route was the only documented case of rabies occurring in pre exposure vaccine completed individual. It is also a fourth cases reported for aerosol transmission of rabies virus. Another point to be noted in this case is he has is neutralizing antibody levels of about 32 IU/ml before 6 months of his infection. This strongly emphasizes that there are no clear cut antibody protection level for rabies prevention. The level that is quoted by WHO is to measure the adequate immune response.

TREATMENT OPTIONS:

1. RABIES VACCINES
2. RABIES IMMUNOGLOBULIN
3. KETAMINE
4. INTERFERON – α
5. RABIES MONOCLONAL ANTIBODIES
6. CORTICOSTEROIDS

RABIES VACCINES:

In animals survival from rabies encephalitis is associated with immune response. So in order to stimulate the immune response both humoral and cell mediated rabies vaccination was tried in human rabies patients but there was no beneficial effects observed. Since immune response after vaccination via intramuscular route vaccines were given via intradermal route at multiple sites for faster immune response but this also results in no beneficial effects. The fault may lies in the fact that attenuated vaccines or recombinant vaccines are for animal vaccination which leads to the stimulation of cytotoxic T cells whereas in case of human beings inactivated rabies vaccines are used and it does not leads to the stimulation of cytotoxic T cells . Till today there was no live attenuated or recombinant anti rabies vaccines were licensed for human use.

ANTIRABIES IMMUNOGLOBULIN:

This is based the hope that rabies immunoglobulin would promote the clearance of the rabies virus but it fails in clinical experiments. The full dose of immunoglobulin is given via intramuscular route in the human rabies cases because the virus has already gained the entry into the nervous system so local infiltration will not be much useful in those cases. It was once believed that higher than dose required for the post exposure prophylaxis was needed for the treatment of human rabies cases. But it also failed to achieve the positive results.

There is also concern thatimmunoglobulins will not enter the blood brain barrier when given via intramuscular route. But the effects and safety concerns of anti-rabies immunoglobulin via intra thecal route was unknown. There are reports that administration via intra thecal route will cause severe brain edema which could results in serious immediate adverse outcomes.

MONOCLONAL ANTIBODIES:

In experimental animal studies it was shown that rabies **MONOCLONAL ANTIBODY 1112-1** clears the virus from the central nervous system when administered before the development of clinical symptoms. It thus leads to the survival of experimentally infected animals. These studies were carried out in rodent model. This shows promising but

human monoclonal antibody or humanized will be a better than mouse monoclonal antibodies. In future this holds promising. As of now it is still in the experimental stages only.

RIBAVIRIN:

1-β-D-Ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide. It is a purine analogue. It is also a RNA mutagen. By acting as a template for cytidine and uridine it induces mutations in the viral genome. It also has immunomodulatory properties. It also demonstrates in vitro activity against the rabies virus. But in vivo efficacy is still under debate. It is a broad spectrum antiviral agent.

It is given with both loading and maintenance dose via intravenous route. It will enter the intact blood brain barrier which is evidenced in animal studies. But in cases of infection the barrier appears to be not intact and there is uptake of ribavirin across the barrier. In order to overcome this uncertainty ribavirin can be given via intra thecal route by means of OMMAYA reservoir. Combined intravenous and intra thecal route also tried but apparently with no benefit.

INTERFERON-α:

It is immunoregulatory protein. It is used in both viral infections as well as in neoplasms. It creates an intracellular environment that interferes with replication of the viruses. It also interacts with the innate immune system and aid in the antigen presentation and also in the activation of cytotoxic T cells. It may also interact synergically with the antibodies. Experiment studies in the monkeys have demonstrated the beneficial effects. But if fails to reproduce in human beings.

KETAMINE:

It is a dissociative anesthetic drug. It causes both sedation and analgesia. It also rapidly causes the blood brain barrier. It also a non-competitive antagonist at NMDA receptor.

There also evidence based on animal studies that ketamine has got in vitro antiviral activity by inhibiting viral replication. It does it by inhibiting viral genome transcription. Based on these it was proposed that NMDA receptors may act as a receptor for rabies virus. But it fails to show consistent results.

CORTICOSTEROIDS:

In animal studies it was found to increase the mortality rates and it also shortens the incubation periods. Based on the above findings is not recommended for the management of severe brain edema, which by itself is a very rare finding due to rabies per se. Therefore it not recommended in the aggressive line of management of human rabies except in faces of adrenal insufficiency. Moreover it closes the blood brain barrier preventing the entry of other drugs.

MILWAUKEE PROTOCOL:

It consist of the following steps

1. Intubation and mechanical ventilation support
2. Drug induced coma
    - Ketamine at doses of 48mg/kg/day in continuous intra venous infusion.
    - Midazolam infusion for 7 days
3. Depending upon the EEG findings to suppress the bursts phenobarbital can be used
4. Intravenous ribavirin
5. Amantadine 200 mg per day via enteral route

The basis is to use neuroprotective agents and maintain life supports and wait for virus neutralizing antibodies to clear the rabies virus from the system. Even though the treatment appears to be beneficial it fails to produce similar results in the clinical practice.

In the next tabular column the documented treatment failures of patients treated with MILWAUKEE protocol are summarized.

Cases of Human Rabies with Treatment Failures that Used the Main Components of the "Milwaukee Protocol"

Case No.	Year of Death	Age and Sex of Patient	Virus Source	Country	Reference
1	2005	47 male	kidney and pancreas transplant (dog)	Germany	(Maier et al., 2010)
2	2005	46 female	lung transplant (dog)	Germany	(Maier et al., 2010)
3	2005	72 male	kidney transplant (dog)	Germany	(Maier et al., 2010)
4	2005	unknown	dog	India	(Bagchi, 2005)
5	2005	7 male	vampire bat	Brazil	–[a]
6	2005	20–30 female	vampire bat	Brazil	–[a]
7	2006	33 male	dog	Thailand	(Hemachudha et al., 2006)
8	2006	16 male	bat	USA (Texas)	(Houston Chronicle, 2006)
9	2006	10 female	bat	USA (Indiana)	(Christenson et al., 2007)
10	2006	11 male	dog (Philippines)	USA (California)	(Aramburo et al., 2011; Christenson et al., 2007)
11	2007	73 male	bat	Canada (Alberta)	(McDermid et al., 2008)

(Continued)

Case No.	Year of Death	Age and Sex of Patient	Virus Source	Country	Reference
12	2007	55 male	dog (Morocco)	Germany	(Drosten, 2007)
13	2007	34 female	bat (Kenya)	The Netherlands	(van Thiel et al., 2009)
14	2008	5 male	dog	Equatorial Guinea	(Rubin et al., 2009)
15	2008	55 male	bat	USA (Missouri)	(Pue et al., 2009; Turabelidze et al., 2009)
16	2008	8 female	cat	Colombia	(Juncosa, 2008)
17	2008	15 male	vampire bat	Colombia	(Badillo et al., 2009)
18	2009	37 female	dog (South Africa)	Northern Ireland	(Hunter et al., 2010)
19	2009	42 male	dog (India)	USA (Virginia)	(Troell et al., 2010)
20	2010	11 female	cat	Romania	(Luminos et al., 2011)
21	2011	41 female	dog (Guinea-Bissau)	Portugal	(Santos et al., 2012)
22	2011	25 male	Dog (Afghanistan)	USA (Massachusetts)	(Javaid et al., 2012)
23	2012	63 male	brown bat	USA (Massachusetts)	(Greer et al., 2013)
24	2012	9 male	marmoset	Brazil	(NE 10, 2012)
25	2012	41 male	dog (Dominican Republic)	Canada (Ontario)	(Branswell, 2012)
26	2012	29 male	dog (Mozambique)	South Africa	(IAfrica.com, 2012; Times Live, 2012)

[a]*Personal communication from Dr. Rita Medeiros, University of Para, Belem, Brazil.*

CHANGE IN POST EXPOSURE PROPHYLAXIS OVER TIME:

Over the time post exposure vaccination schedule as well as the number of doses required has been changing. This is mainly because of our better understanding of the immune response to rabies vaccination when compared to the past and also because of the more potent vaccine developments. These modern day vaccines are free of major adverse effects when compared to the older vaccines.

Comparative rabies post-exposure vaccination schedules over time (intramuscular [IM] unless indicated as intradermal [ID]).

Year	Vaccine	Doses	Schedule (days)	Source
1973	DEV	14–23	Daily	WHO
1984	HDCV	6	0, 3, 7, 14, 30, 90	WHO
1992	TC/PDEV	5	0, 3, 7, 14, 30	WHO
1992	TC/PDEV	4	0, 0, 7, 21	WHO
1992	TC/PDEV (ID)	8	0, 0,3, 3, 7, 7, 30, 90	WHO
1999	HDCV, PCECV, RVA	5	0, 3, 7, 14, 28	ACIP; WHO
2005	CC/PEEV	5	0, 3, 7, 14, 28	WHO
2005	CC/PEEV (ID)	8	0, 0, 3, 3, 7, 7, 28, 28	WHO
2005	HDCV/PCECV (ID)	14	Day 0 x 8, 7 x 4, 28, 90 x 1	WHO

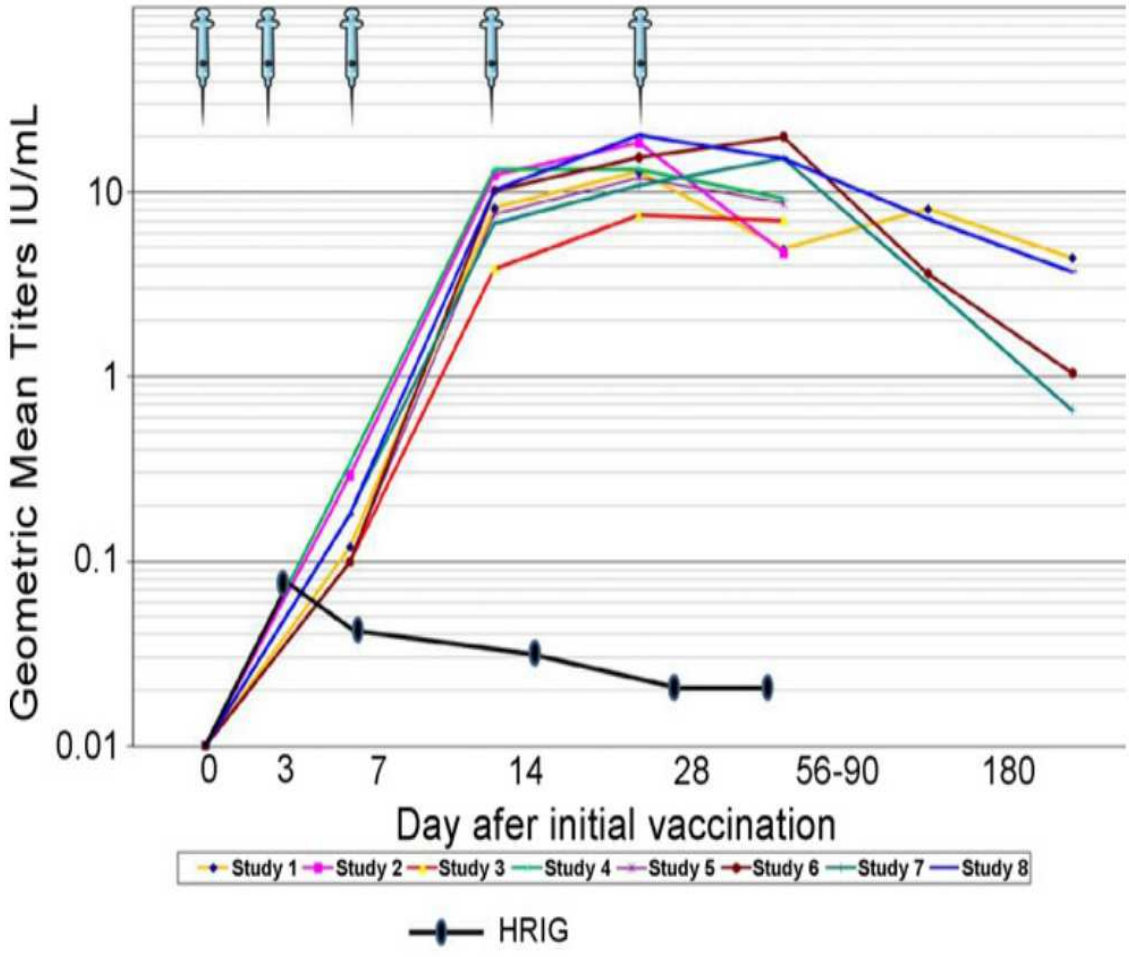
There are also many human studies regarding the antibody levels following post exposure vaccination using various regimens and also with various vaccine preparations. Based on these studies only the center for disease control came with the latest recommendation of reduced four dose intra muscular regimen in 2010. Even though the post exposure vaccine regimens has changed over the course of time. The indication for anti-rabies immunoglobulin has not changed.

Studies of rabies antibody values post-vaccination with various regimens.

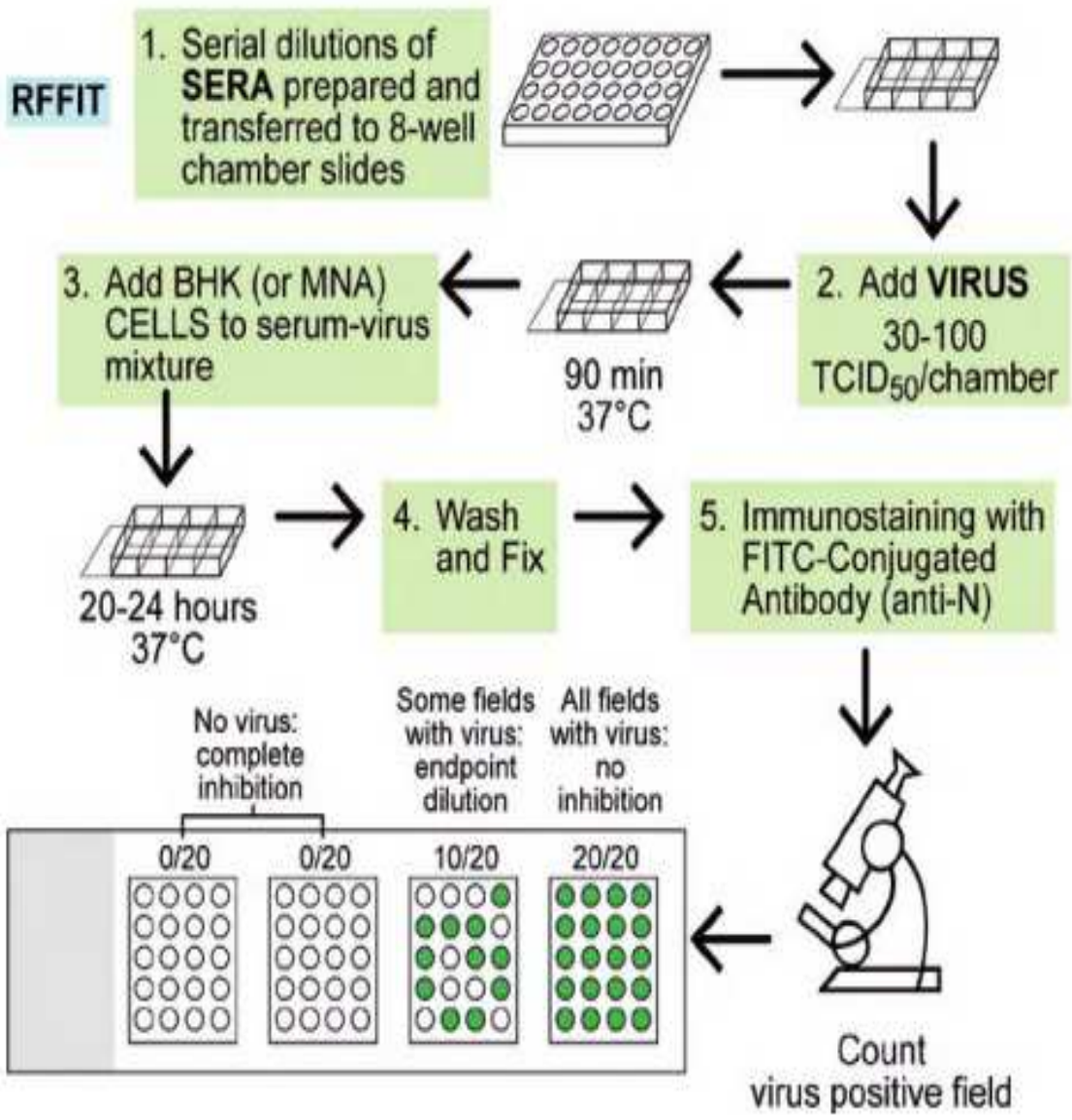
Location	Subjects	Vaccine	Schedule	Findings
Iran	45 persons bitten by rabid dogs or wolves	HDCV	0, 3, 7, 14, 30, 90	All with adequate values after 4 doses; titers decreased after 5th dose
Germany	16 volunteers	HDCV ± RIG	0, 3, 7, 14, 30, 90	All with adequate values by day 14; no interference with RIG; no increase after 5th dose
Manitoba	24 volunteers	HDCV ± RIG	0, 3, 7, 14, 28	All with adequate values after 3 doses; no increase with 4th or 5th dose
Manitoba	Children and adults bitten by suspected rabid animals	HDCV + RIG	0, 3, 7, 14, 28	All with adequate values after 3 doses; no increase following either 4th or 5th dose
Thailand	27 children bitten by rabid animals	PCECV ± RIG	0, 3, 7, 14, 28, 90	All with adequate values after 3 doses
India	37 persons bitten by suspected rabid animals	PVCV	0, 3, 7	All with values ≥1.2 IU/l at 10–15 days after 3rd dose
India	62 persons bitten by suspected rabid animals	PVCV	0, 3, 7, 14 or 0, 7, 14, 30	All with values ≥1.2 IU/l at 10–15 days after 4th dose
Wisconsin	32 adult volunteers	HDCV ± RIG	0, 3, 7, 14, 28	All with adequate values; maximum on day 14; no interference
USA	680 adult volunteers	HDCV or CPRV + RIG	0, 3, 7, 14, 28	All with adequate values by day 14
Thailand	57 persons presenting for RPEP	PCECV ± RIG	0,3,7,14, 30,90	All with adequate values after 4 doses
Nebraska; India	23 adult volunteers	PCECV + RIG (Mabs)	0, 3, 7, 14, 28	All with adequate values by day 14

There are many cases of vaccination failures documented in the literatures, but most of them are due to improper wound management and not following the schedules properly in applying immunoglobulin and as well as in vaccination. True vaccination per se is very rare.

GRAPHICAL REPRESENTATION OF VARIOUS STUDIES IN COMPARING ANTIBOBY TITERS ON DIFFERENT DAYS OF VACCINATION:



RAPID FLUORESCENT FOCUS INHIBITION TEST [RFFIT]:



STEPS INVOLVED IN THE RFFIT:

- The serum to be tested is diluted in a 96 well plate and transferred into a 8 –well chambered slides
- Then the rabies challenge virus is added to the diluted serum
- The slides are then incubated for 90 minutes
- There after mouse neuroblastoma cells are added to each of the wells
- Then the slides are incubated in an incubator for 20 to 24 hours
- The incubator should be incubated at 37^0C and the CO_2 concentration should be 2-5%
- Then the slides are washed and fixed with 80% cold acetone
- Then the FITC conjugated anti rabies virus antibody directed against rabies virus was added
- Then the 20 fields of each well of 8 chambered slides are examined under the fluorescent microscope for the presence of fluorescence. If fluorescence is present it indicates the presence of non-neutralized virus infected cells
- Then the 50% end point titer of serum is determined using REED – MEUNCH formula.
- Then the potency of test serum is calculated in IU/ml using the formula

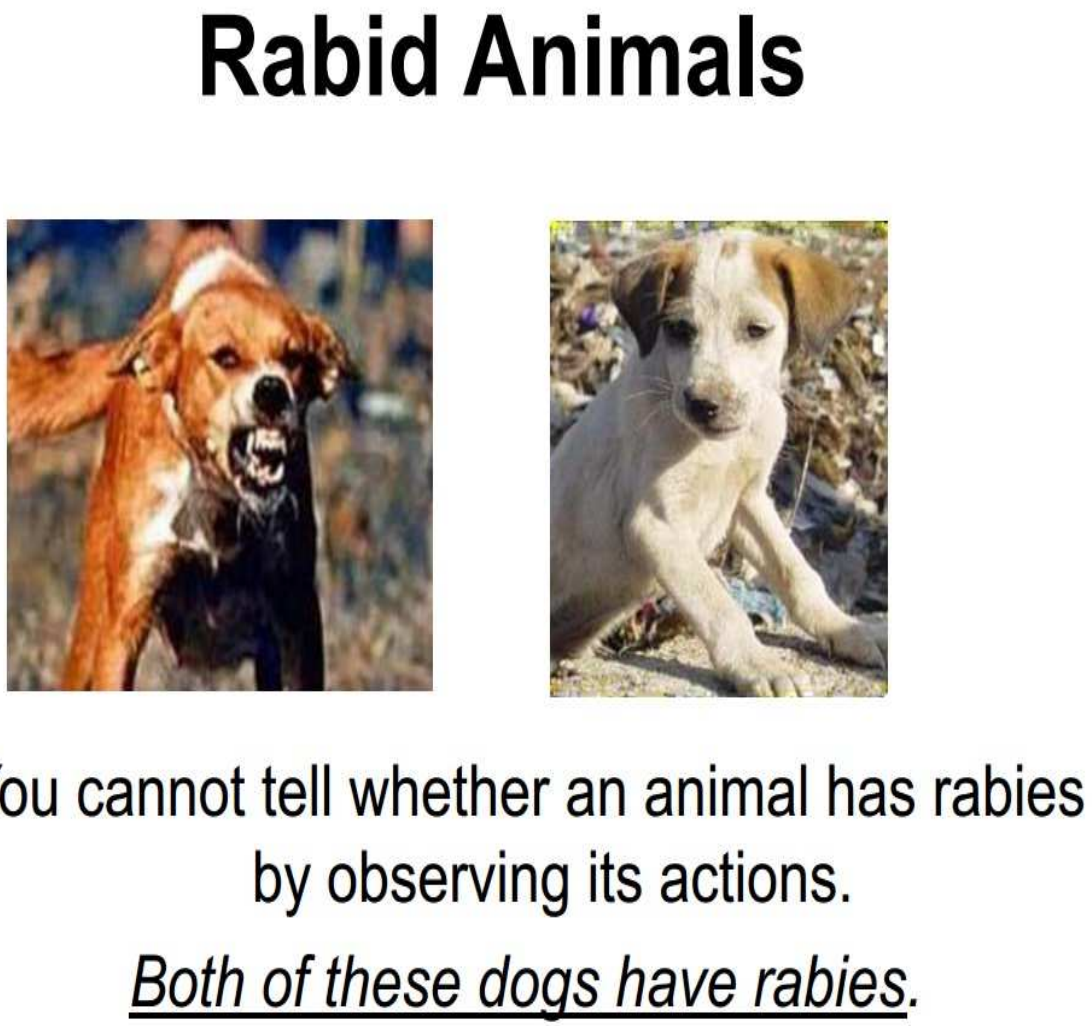
$$\text{Number of IU/ml} = \frac{\text{End-point titer of the test serum}}{\text{End-point titer of the reference}} \times \text{2 IU/ml in reference serum}$$

- **KNOWLEDGE ATTITUDE AND PRACTICE REGARDING DOG BITE:**

Since human rabies is nearly always fatal. The knowledge attitude practice regarding dog bite plays a vital role in the prevention of rabies. There are lots of misbeliefs and false practices regarding dot bites and its management.

Still there are many believing in the tradition practices over medical management for dog bites. But somehow they all seem to know about the observation period of the dogs in case of dog bite but they did not know the reason for observation. Mass media plays a big role in the instillation of these thoughts in their minds. They also think that medical management needed only for the unprovoked bites and for the bites by stray dogs only. Most of seem to me unaware about the transmission of rabies by wild animals and other animals. They also think that post exposure prophylaxis is not necessary for the bite by vaccinated pet dogs. There is also thought individual should observe food restriction for some particular time period. Similarly there is also thought that rabies spreads from human to human.

Many KAP studies have been conducted to assess the knowledge attitude and practices among people regarding pet care, need for pet vaccination, dog bite managements. Surprisingly there is still lack of knowledge regarding dog bite management among treating physicians. This is mainly in the areas of current guidelines followed in the management of animal bites.



MATERIALS AND METHODS

MATERIALS AND METHODS

SETTING:

This study was conducted at the Anti-Rabies Vaccine Clinic, Rajiv Gandhi Government General Hospital and Madras Medical College.

ETHICAL COMMITTEE APPROVAL:

Obtained.

STUDY DURATION:

This study was conducted over a period of six months.

STUDY POPULATION:

Patients coming to anti-rabies vaccine clinic for the post exposure prophylaxis management

SAMPLE SIZE:

100 patients

TYPE OF STUDY:

Observational study

INCLUSION CRITERIA:

- New dog bite individuals with wounds belonging class, requiring both active and passive immunization.

EXCLUSION CRITERIA:

- History of previous dog bite.
- History of anti-rabies vaccination or anti-rabies immunoglobulin in the past.
- Other animal bites.
- Clinical suspicion of rabies.

DATA COLLECTION AND METHODS

Informed consent will be obtained from all the patients participating in the study.

Patients have their history taken regarding the dog bite and then subjected to clinical examination of the wounds.

The patients were given equine immunoglobulin at the dose of 40IU/kg locally infiltrated around the wound. Remaining if any is given at a distant site via intramuscular route.

Then they are given 5 doses of 0.5ml of purified vero cell vaccine via intramuscular route on day 0, 3, 7, 14, and 28. The day ‘0’ being the day of vaccination and the day of dog bite. This regimen is approved by government of India

The vaccine used in our hospital is ABHAYRAB – purified vero cell vaccine the anti-rabies immunoglobulin used in our hospital is EQUIRAB – equine anti rabies immunoglobulin.

Then on the 14th day when the patient comes for the fourth dose of vaccination 5ml of venous blood sample is obtained prior to the fourth dose of vaccination. Then on the 21st day 5ml of venous sample is obtained. Each date of blood sampling collection corresponds to 7 days after respective doses of vaccination.

Soon after the blood sample collection serum is separated under sterile aseptic precautions and then the sample is stored under -20^{0}C. After the collection samples from all 100 patients [totally 200 samples], serum anti rabies virus neutralizing antibody titers are measured using Rapid Fluorescent Focus Inhibition Test [RFFIT].

All the data will be entered in the Performa [enclosed].

Data will be analyzed using SPSS package and ANOVA.

To the same individuals questionnaire comprising of 10 questions were given to assess their knowledge, attitude and practice regarding dog bite were given. Each correct response carries '1' mark while the wrong question carries '0' mark. The questionnaire consist of 5 questions assessing their knowledge and another 5 questions assessing their attitude and practice.

All the data will be entered in the Performa [enclosed].

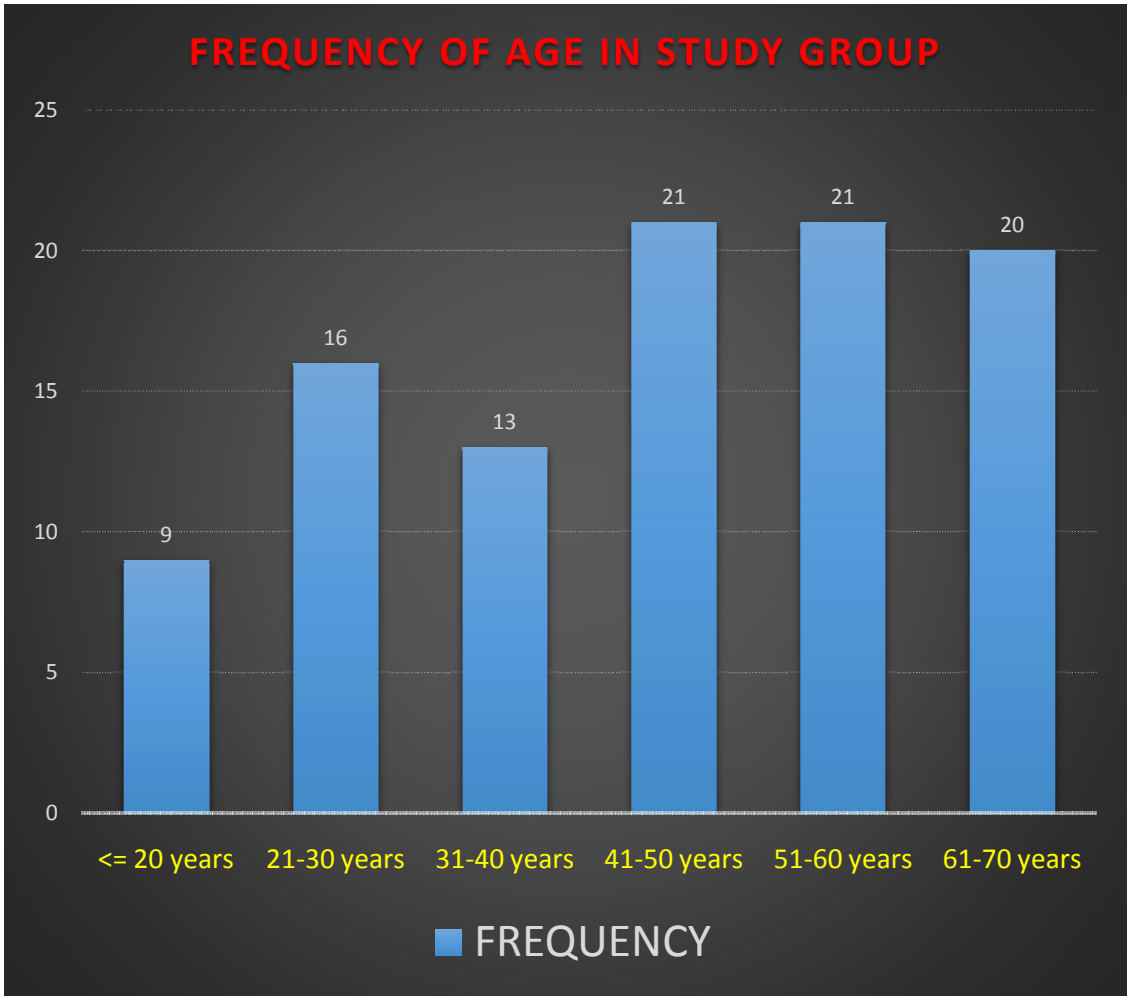
Data will be analyzed using SPSS package and ANOVA.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

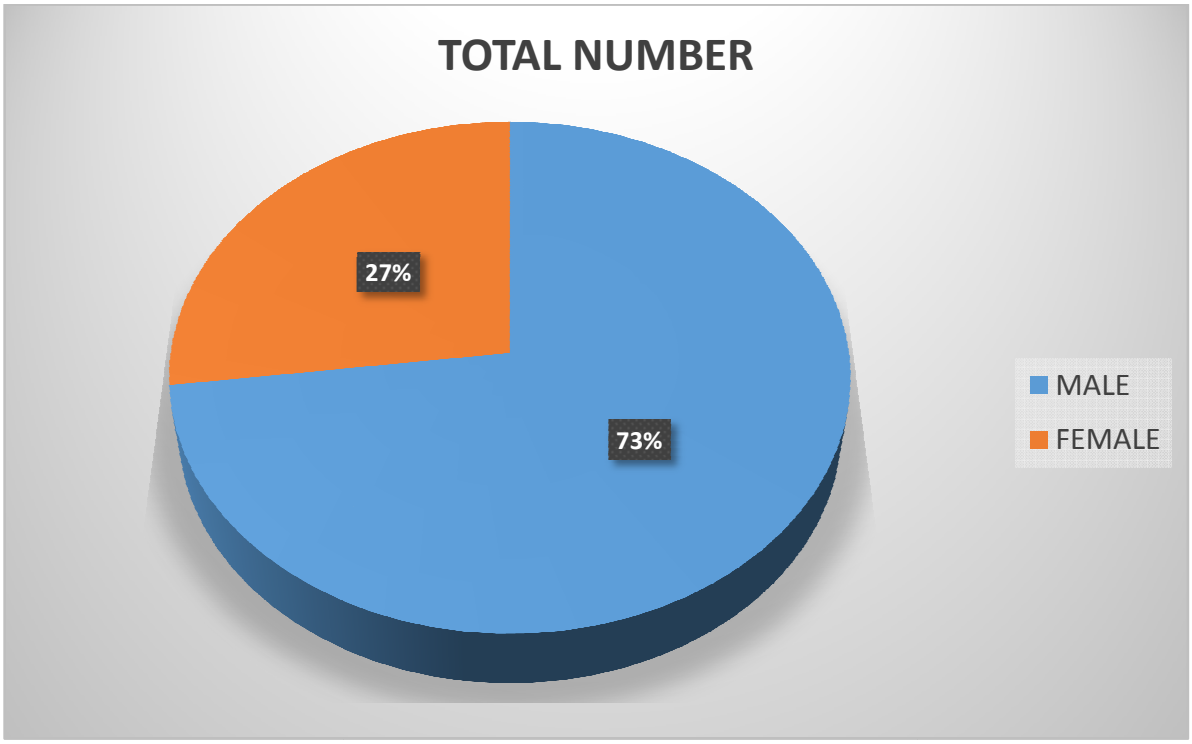
AGE DISTRIBUTION IN THE STUDY GROUP:

Age in years	Frequency	Percent	Valid Percent	Cumulative Percent
<= 20	9	9.0	9.0	9.0
21-30	16	16.0	16.0	25.0
31-40	13	13.0	13.0	38.0
41-50	21	21.0	21.0	59.0
51-60	21	21.0	21.0	80.0
61-70	20	20.0	20.0	100.0
Total	100	100.0	100.0	


FREQUENCY OF AGE IN STUDY GROUP
25
20
15
10
5
0
9
16
13
21
21
20
<= 20 years
21-30 years
31-40 years
41-50 years
51-60 years
61-70 years
FREQUENCY

SEX DISTRIBUTION IN STUDY GROUP:

Gender	Frequency	Percent	Valid Percent	Cumulative Percent
Male	73	73.0	73.0	73.0
Female	27	27.0	27.0	100.0
Total	100	100.0	100.0	



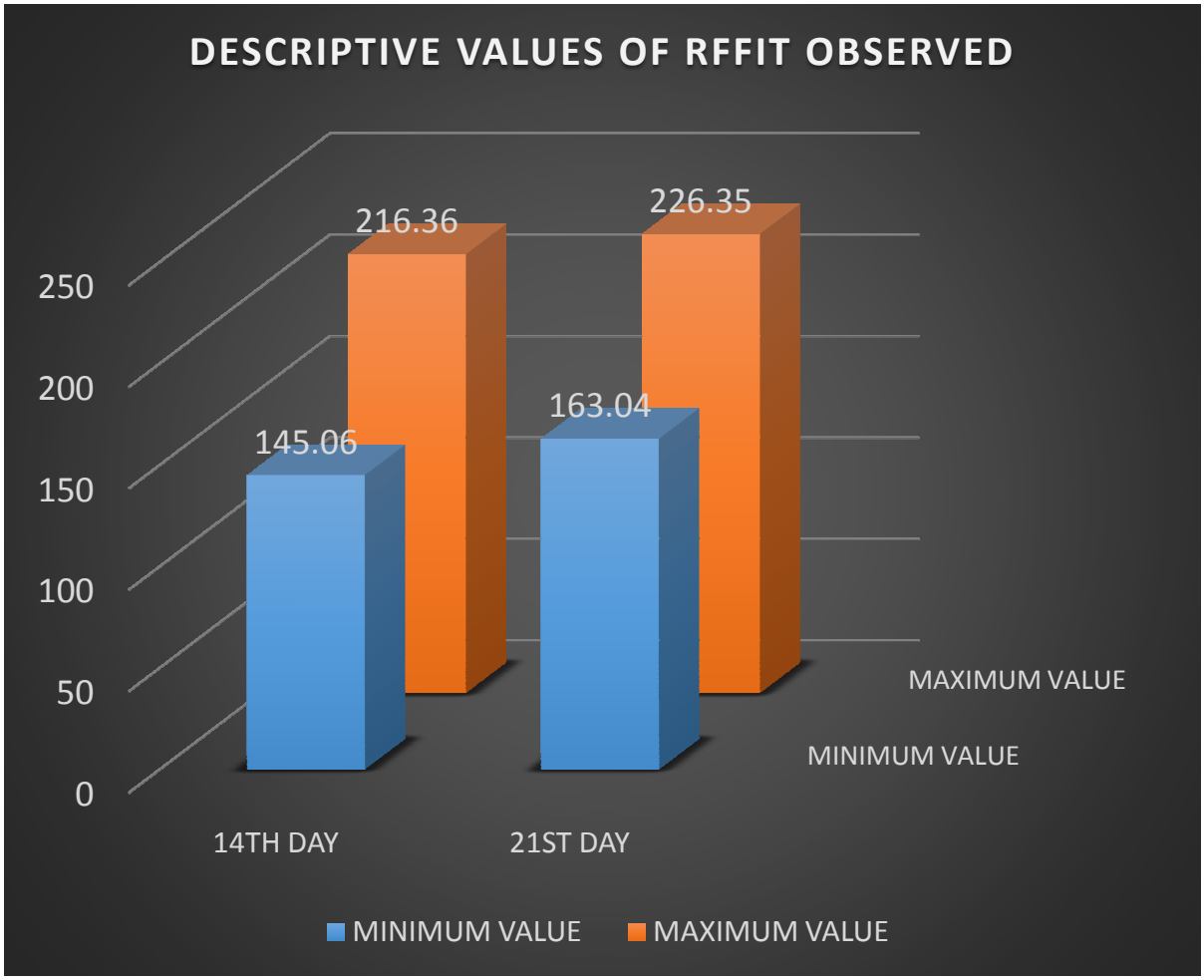
DESCRIPTIVE STATISTICS

RFFIT VALUES	Number Of Individuals	Minimum	Maximum	Mean	Std. Deviation
AT 14^{TH} day	100	145.06	216.36	176.4822	17.29897
AT 21^{ST} day	100	163.04	226.35	200.6443	14.32372

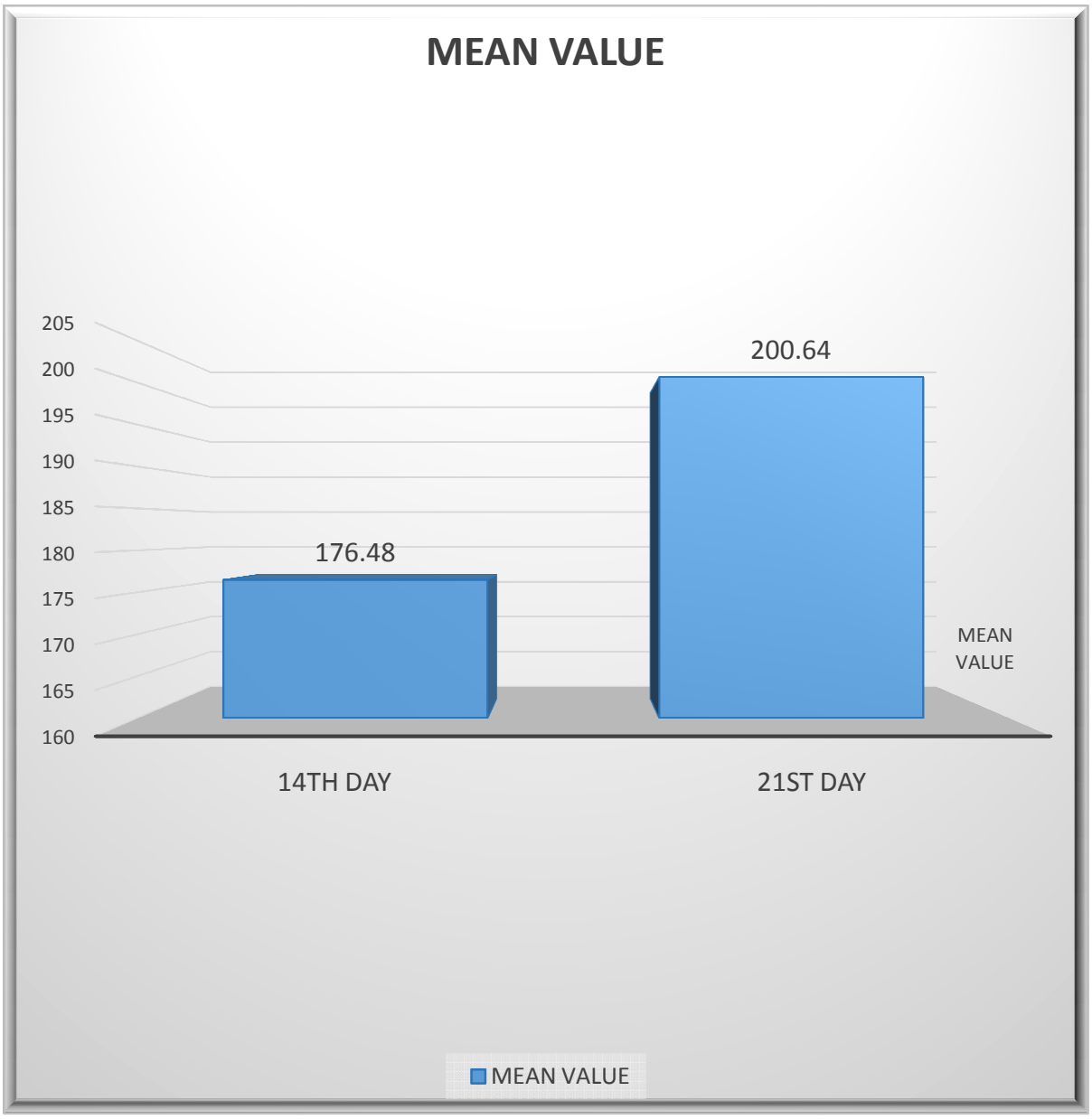
Paired Samples Statistics: T-Test

RFFIT VALUES AT	Mean	Number OF individuals	Std. Deviation	Std. Error Mean	'p' value
14^{th} day	176.4822	100	17.29897	1.72990	<0.001 (HIGHLY SIGNIFICANT)
21^{st} day	200.6443	100	14.32372	1.43237	

DESCRIPTIVE VALUES OF RFFIT



MEAN RFFIT VALUES OBSERVED IN THE STUDY GROUP:



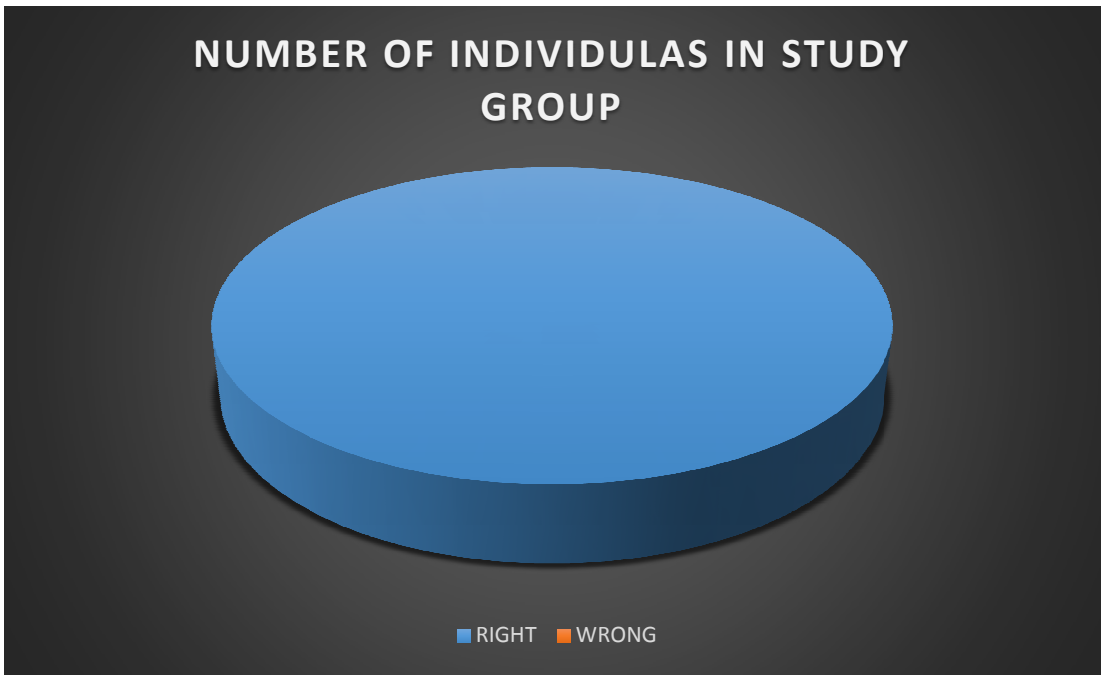
FREQUENCY TABLE:

KNOWLEDGE QUESTIONNAIRE 1:

WHAT TREATMENT OPTIONS ARE AVAILABLE FOR DOG BITE?

ANSWERS : Post exposure prophylaxis in the form of passive and active immunization.

RESPONSE	Frequency	Percent	Valid Percent	Cumulative Percent
Right	100	100.0	100.0	100.0

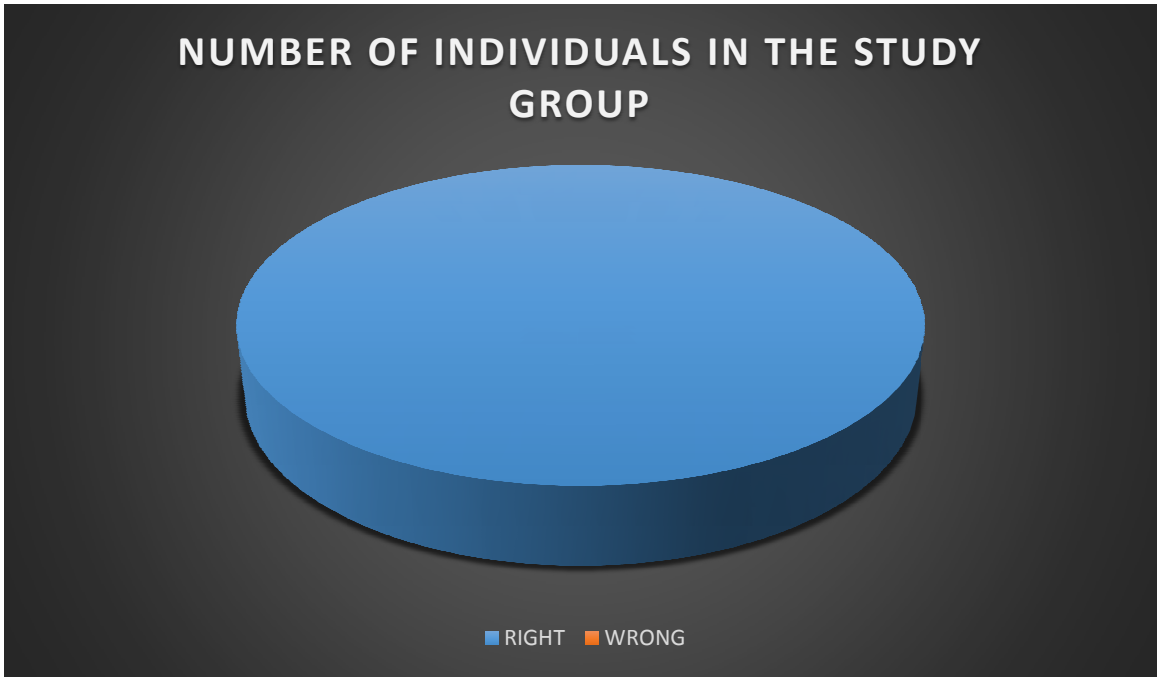


KNOWLEDGE QUESTIONNAIRE 2:

WHAT IS THE MODE OF TRANSMISSION OF RABIES?

ANSWERS: Through the bite of rabid animals.

RESPONSE	Frequency	Percent	Valid Percent	Cumulative Percent
Right	100	100.0	100.0	100.0

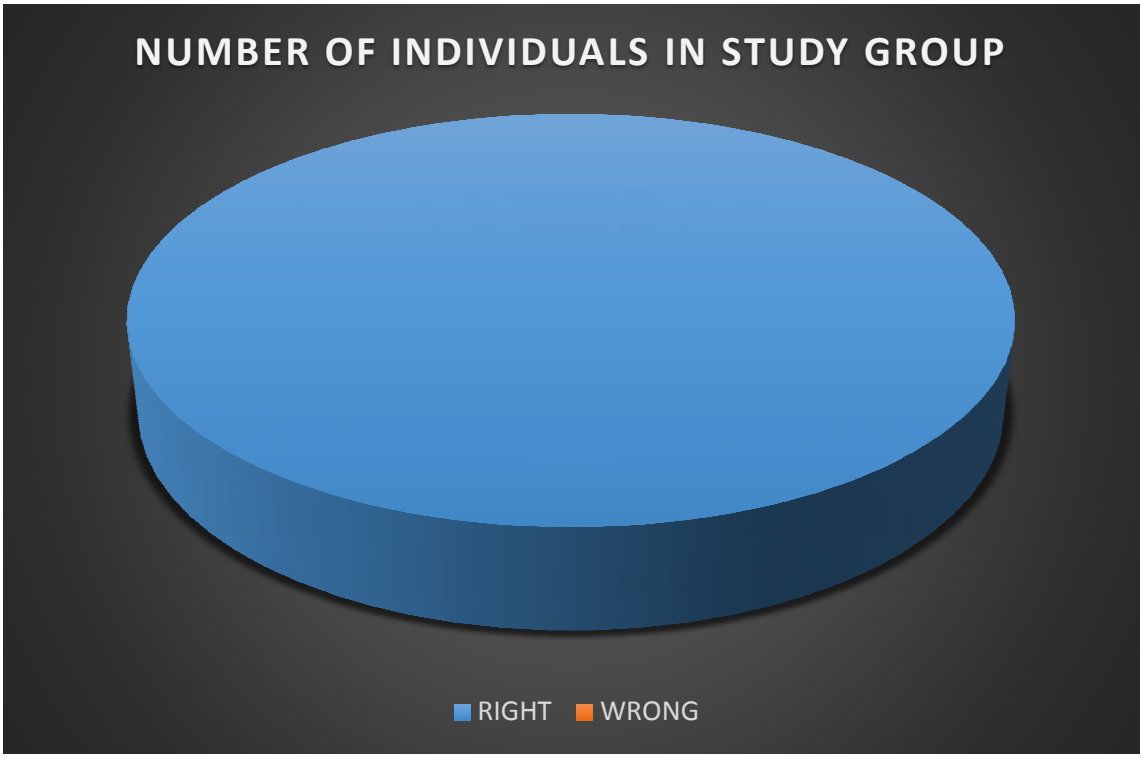


KNOWLEDGE QUESTINNAIRE 3:

HOW MANY DAYS ONE SHOULD OBSERVE THE DOG FOLLOWING BITE AND WHAT FOR?

ANSWER: 10 DAYS. TO OBSERVE IF THE DOG DEVELOPS ANY SIGHS OF RABIES OR DIE WITHIN TEN DAYS DUE TO RABIES.

RESPONSE	Frequency	Percent	Valid Percent	Cumulative Percent
Right	100	100.0	100.0	100.0

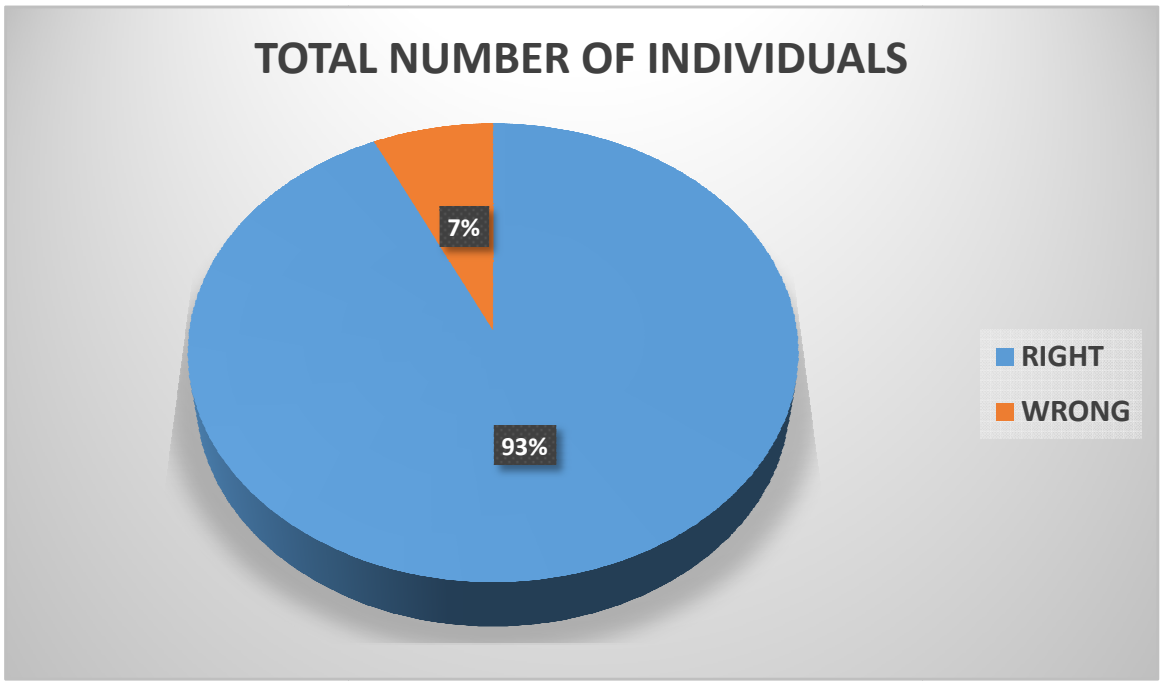


KNOWLEDGE QUESTIONNAIRE 4:

CHANCES OF SURVIVAL IF RABIES DEVELOPS?

ANSWER: AT PRESENT NO.

RESPONSE	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
RIGHT	93	93	93	93
WRONG	7	7	7	7

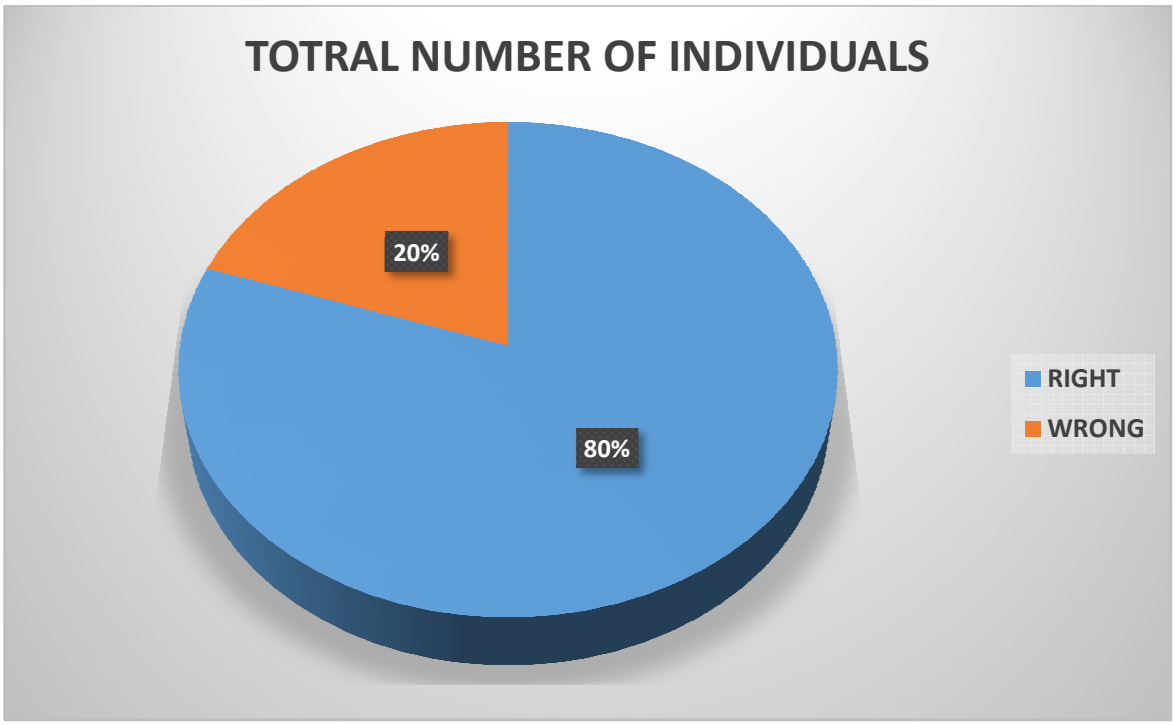


KNOWLEDGE QUESTIONNAIRE 5:

WILL RABIES SPREAD FROM HUMAN TO HUMAN?

ANSWER: NO.

RESPONSE	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
RIGHT	13	13	13	13
WRONG	87	87	87	87

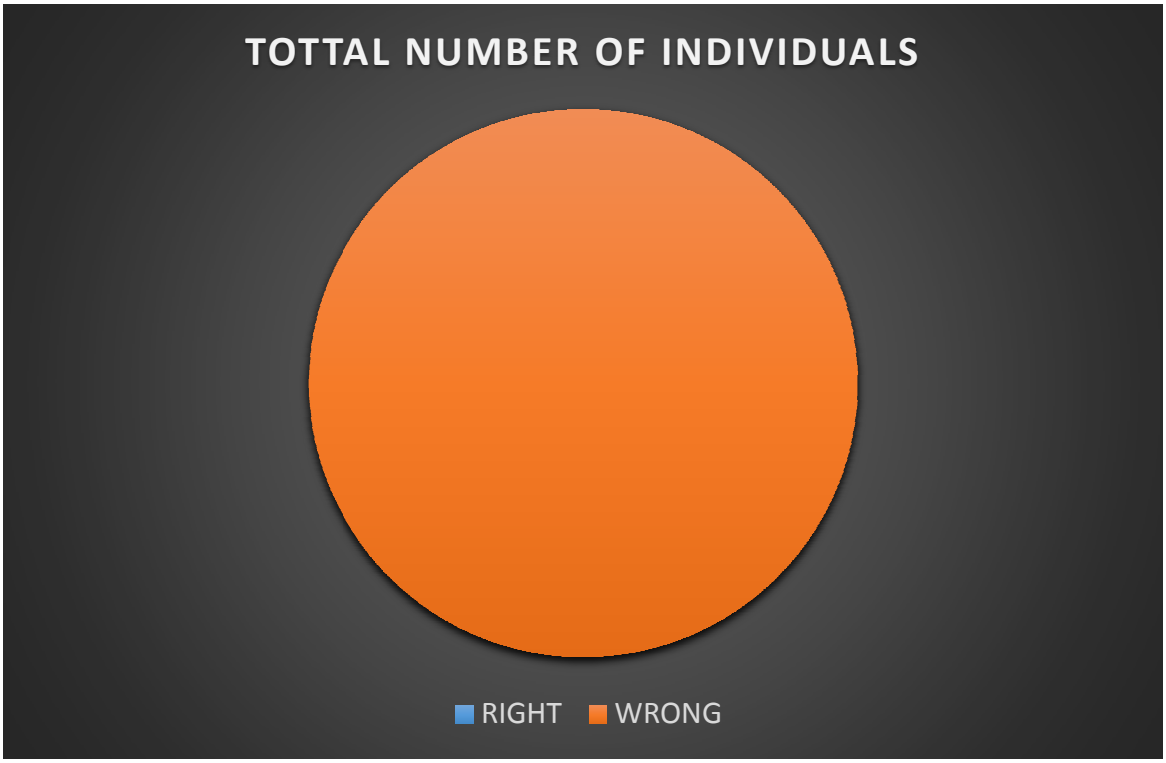


ATTITUDE AND PRACTICE QUESTONNAIRE 1:

YOUR OPINION REGARING WOUND WASHING AND IMMEDIATE SUTURING?

ANSWER: WOUND SHOULD BE WASHED WITH PLENTY OF WATER FOR 15 MINUTES AND THEN WITH IODINE OR ALCOHOL. WOUND SHOULD NOT BE SUTURED IMMEDIATELY.

RESPONSE	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
RIGHT	100	100	100	100

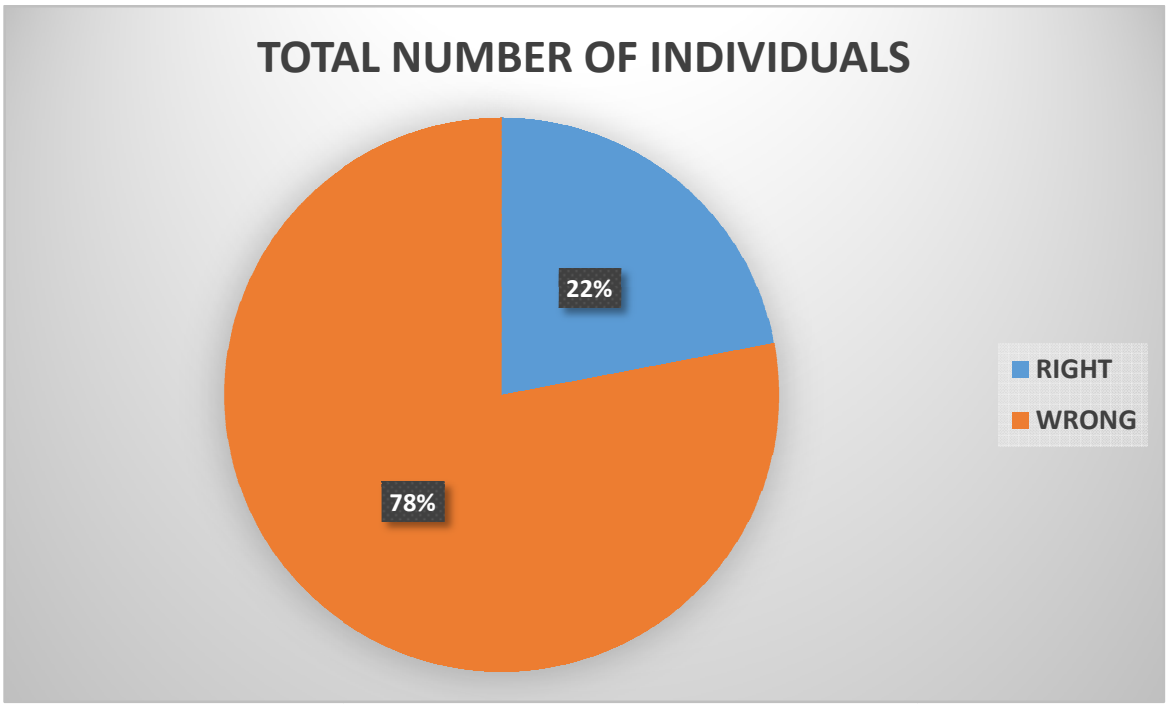


ATTITUDE AND PRACTICE QUESTONNAIRE 2:

YOUR OPINION REGARDING PROPHYLAXIS IN CASE OF PROVOKED DOG BITE?

ANSWER: YES, POST EXPOSURE PROPHYLAXIS SHOULD BE GIVEN.

RESPONSE	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
RIGHT	22	22	22	22
WRONG	78	78	78	78

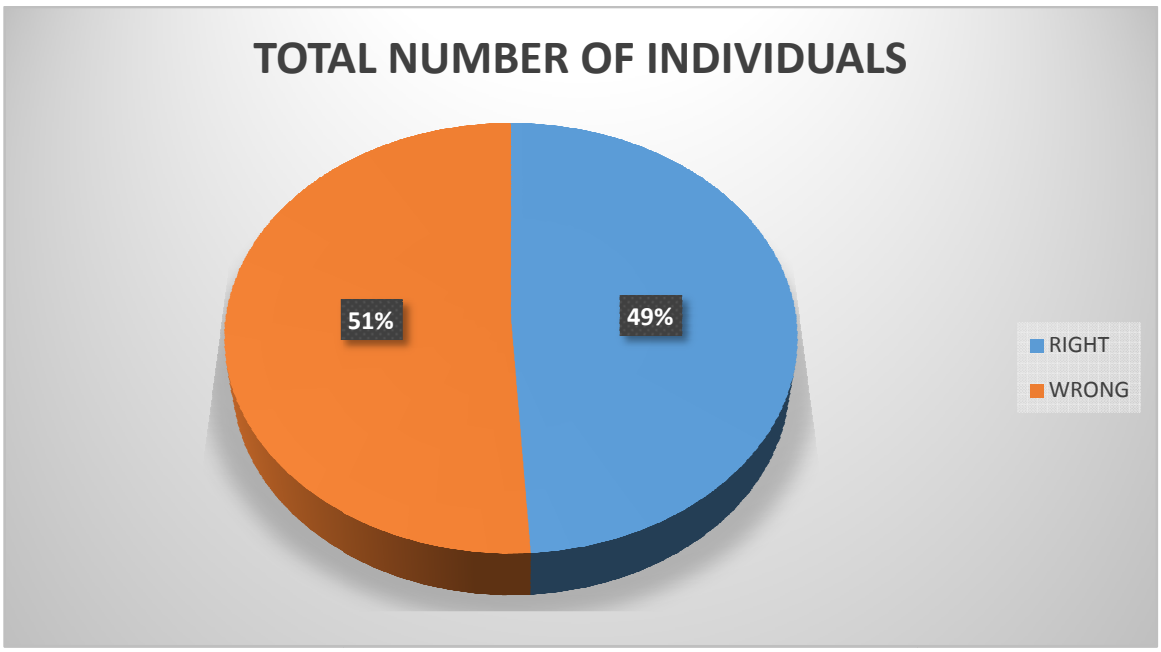


ATTITUDE AND PRACTICE QUESTONNAIRE 3:

WHETHER ALL DOSES OF ARV [ANTI RABIES AVCCINATION] IN THE PEP [POST EXPOSURE PROPHYLAXIS] SCHEDULE SHOULD BE COMPLETED?

ANSWERS: YES, IT SHOULD BE COMPLETED ACCORDING TO THE REGIMEN

RESPONSE	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
RIGHT	49	49	49	49
WRONG	51	51	51	51

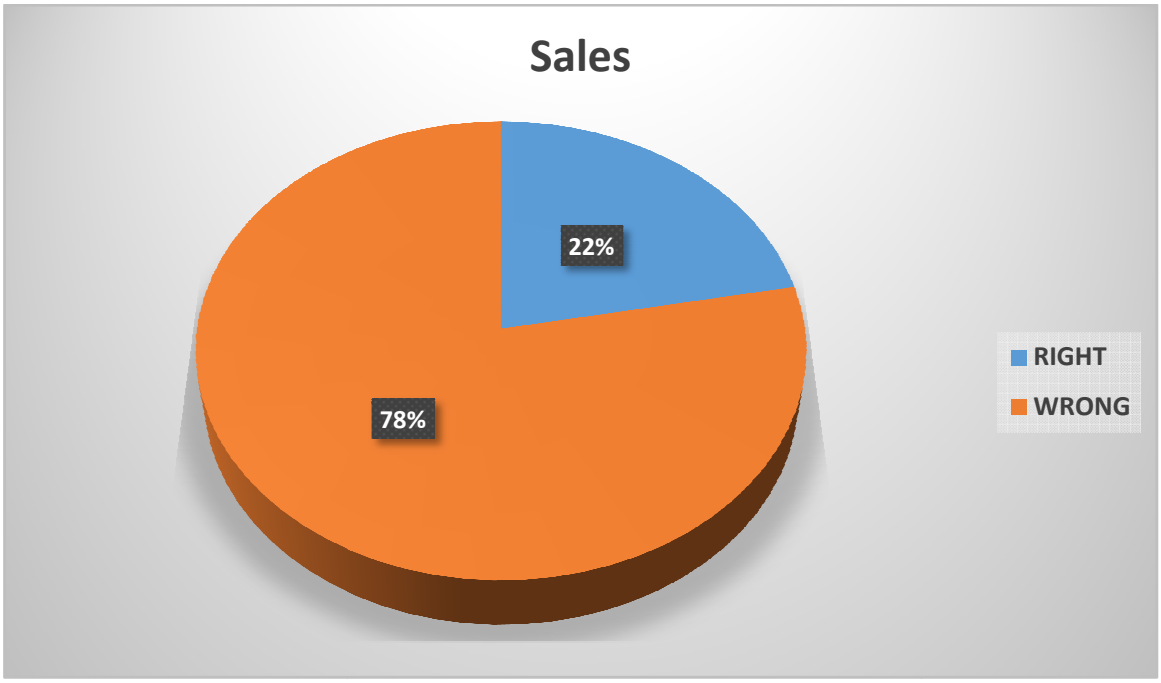


ATTITUDE AND PRACTICE QUESTONNAIRE 4:

YOUR OPINON WHETHER PEP [POST EXPOSURE PROPHYLXIS] ARV [ANTI RABIES VACCINATION] NEEDED IN VACCINATED DOG BITES?

ANSWERS: YES

RESPONSE	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
RIGHT	22	22	22	22
WRONG	78	78	78	78

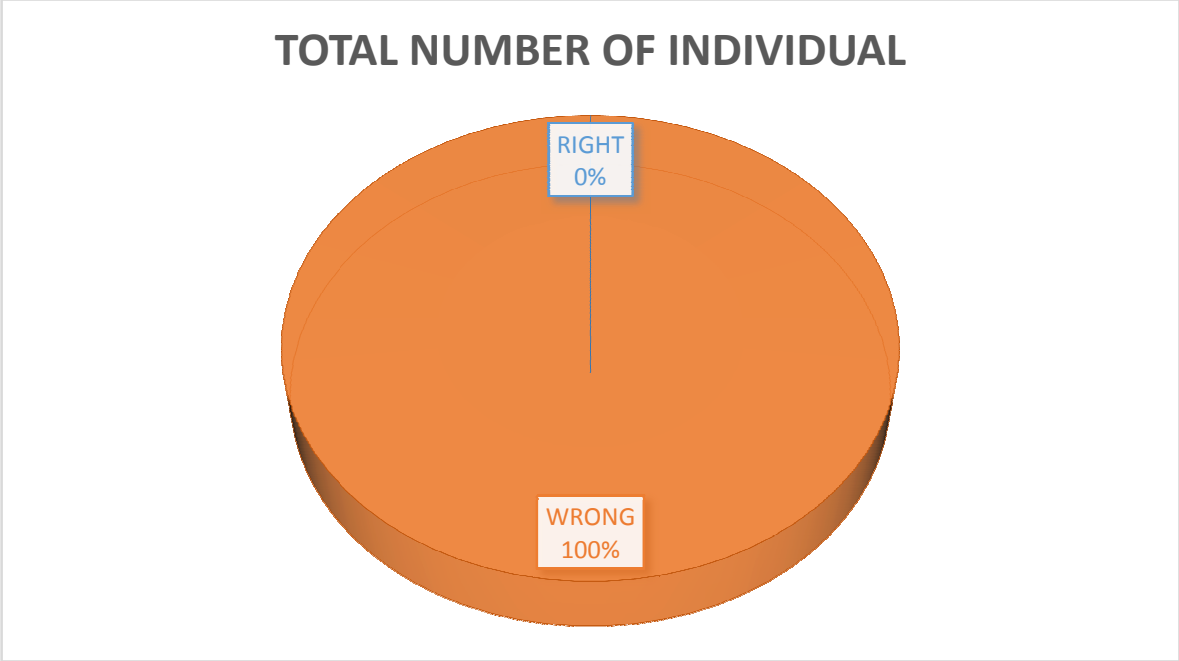


ATTITUDE AND PRACTICE QUESTONNAIRE 5:

WHETHER AVOIDENCE OF CERTAIN FOODS NEEDED AFTER DOGBITE FOR ANY PARTICULAR PERIOD?

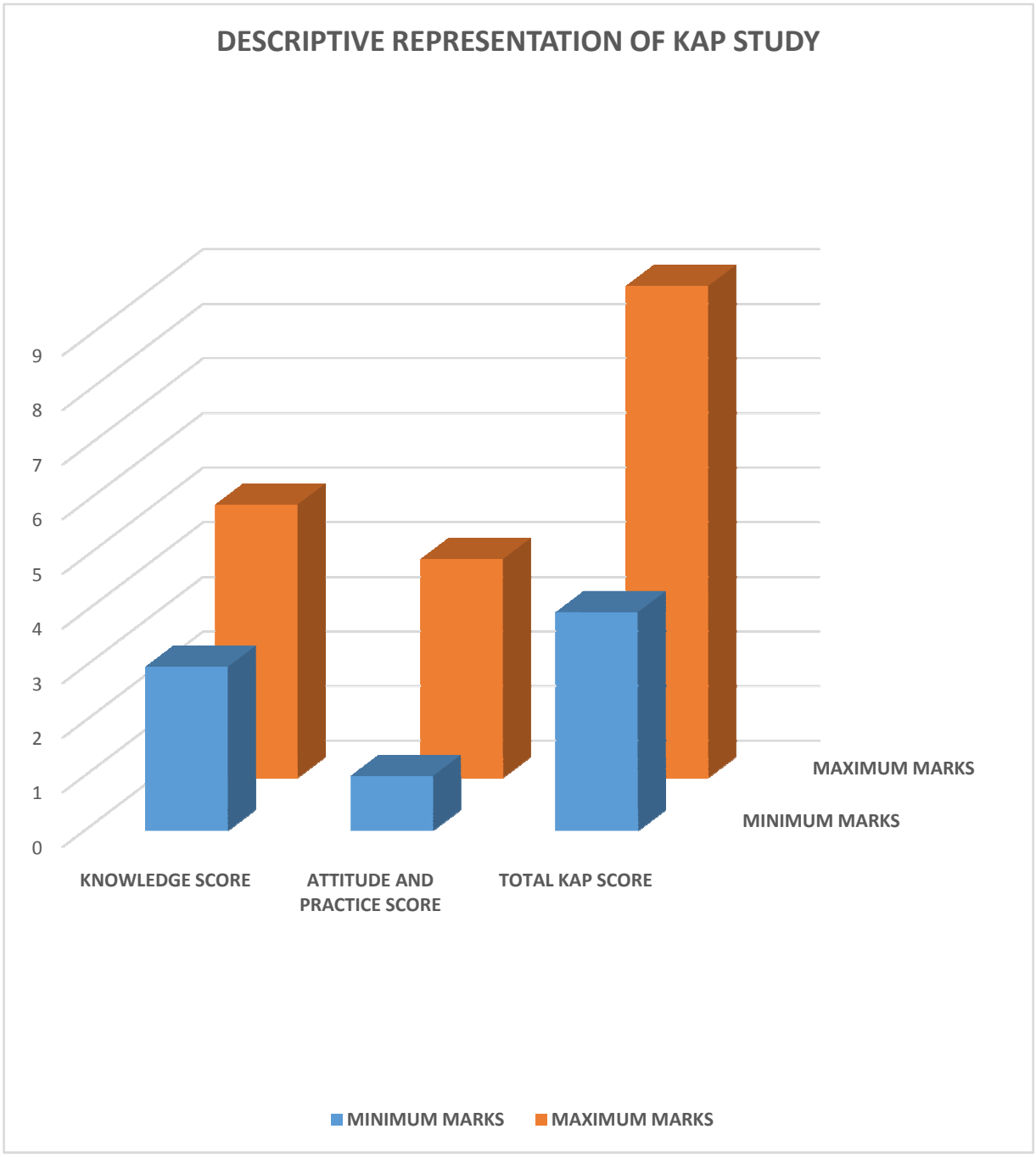
ANSWER: NO

RESPONSE	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
WRONG	100	100	100	100



DESCRIPTIVE STATISTICS FOR KAP ANALYSIS:

	NUMBER OF INDIVIDUALS	MINIMUM MARKS	MAXIMUM MARKS	MEAN	STANDARD DEVIATION
KNOWLEDGE SCORE	100	3	5	4.06	0.422
ATTITUDE AND PRACTICE SCORE	100	1	4	1.93	0.935
TOTAL KAP SCORE	100	4	9	5.99	1.000


DESCRIPTIVE REPRESENTATION OF KAP STUDY
9
8
7
6
5
4
3
2
1
0
KNOWLEDGE SCORE
ATTITUDE AND PRACTICE SCORE
TOTAL KAP SCORE
MAXIMUM MARKS
MINIMUM MARKS
MINIMUM MARKS
MAXIMUM MARKS

SEX BASED DESCRIPTIVE STATISTICS FOR KAP SCORE:

	SEX	NUMBER OF INDIVIDUALS	MEAN	STANDARD DEVIATION	STANDARD ERROR MEAN	'p' VALUE
KNOWLEDGE SCORE	MALE	73	4.05	0.437	0.51	0.303 INSIGNIFICANT
	FEMALE	27	4.07	0.385	0.74	
ATTITUDE AND PRACTICE SCORE	MALE	73	1.97	0.912	0.107	0.054 INSIGNIFICANT
	FEMALE	27	1.81	1.001	0.193	
TOTAL KAP SCORE	MALE	73	6.03	0.986	0.115	0.124 INSIGNIFICANT
	FEMALE	27	5.89	1.050	0.202	

RESULTS

AGE DISTRIBUTION:

In our study 9% of the individual are between 12 – 20 years. 16% of the individual are between 21-30 years. 13% of the individual are between 31-40 years. 21% of individuals are between are between 41- 50 years. 21% of individuals are between 51-60 years. 20 % individuals are between 61-70 years. The mean age in our study group is 44 years.

SEX DISTRIBUTION:

In our study group males were 73% and females are 27%.

CORRELATION BETWEEN ANTIBODY TITERS ON 14TH DAY AND 21ST DAY:

The minimum titer of anti-rabies virus neutralizing antibody observed on 14th day is 145.06 IU/ml and the maximum antibody titer observed on 14th day is 216.36 IU/ml. The average antibody titer observed on 14th day is 176.48 IU/ml

The minimum titer of anti-rabies virus neutralizing antibody observed on 21st day is 163.04 IU/ml and the maximum antibody titer observed on 21stday is 226.35 IU/ml. the average antibody titer observed on 21st day is 200.64 IU/ml

When compared with 14th day [7 days after the third dose of intramuscular vaccination] the antibody titer rise on 21st day [7 days after fourth dose of intramuscular vaccination] is highly significant ['p' value <0.001].

KNOWLEDGE ATTITUDE AND PRACTICE ANALYSIS:

KNOWLEDGE QUESTIONNAIRE:

QUESTION 1:

100% of individuals gave the correct response.

QUESTION 2:

100% of individuals gave the correct response.

QUESTION 3:

100% of individuals gave the correct response.

QUESTION 4:

93% of individuals gave the correct response.

7% of individuals gave the wrong response.

QUESTION 5:

13% of the individuals gave the correct response

87% of the individuals gave the wrong response.

ATTITUDE AND PRACTICE QUESTIONNAIRE:

QUESTION 1:

100% of the individuals gave the correct response.

QUESTION 2:

22% of the individual gave the correct response.

78% of the individual gave the wrong response.

QUESTION 3:

49% of the individual gave the correct response.

51% of the individual gave the wrong response.

QUESTION 4:

22% of the individual gave the correct response.

78% of the individual gave the wrong response.

QUESTION 5:

0% of the individual gave the correct response.

100% of the individuals gave the wrong response.

COMPARISON OF KNOWLEDGE ATTITUDE AND PRACTICE WITH AGE AND SEX DISTRIBUTION:

In the study group regarding knowledge questionnaire the minimum mark obtained by an individual is 3 out of 5. While the maximum mark obtained by an individual is 5 out of 5. The study group has an average of 4.06 out of 5.

In the study group regarding attitude and practice questionnaire the minimum mark obtained by an individual is 1 out of 5. While the maximum mark obtained by an individual is 4 out of 5. The study group has an average of 1.93 out of 5.

The knowledge, attitude and practice regarding dog bite between sex and age distribution is INSIGNIFICANT [‘p’ value of 0.124].

In our study of knowledge attitude and practice regarding dog bite, knowledge was adequate but the attitude and practice were inadequate and should be improved**.**

DISCUSSION

DISCUSSION

ALL THE STUDIES QUOTED BELOW HAS USED THE ANTI RABIES VACCINATION VIA INTRAMUSCULAR ROUTE.

- In a study conducted in IRAN by BAHMANYAR et al on 45 persons bitten by rabid dogs and wolves human diploid cell vaccine was used. The vaccines were give as 6 doses on day 0, 3, 7, 14, 28 and 90. They observed that all develop adequate levels of antibodies after 4 doses. No immunoglobulin was used in the study. This was contrast to our study as our study group develop adequate antibodies after the third dose itself.

- In a study conducted in GERMANY by KUWERT et al on 16 volunteers using human diploid cell vaccine and immunoglobulin, the vaccines were given as 6 doses on day 0, 3, 7, 14, 28 and 90. They all develop adequate levels on day 14. This correlates with our study results. That our study group individuals develop adequate levels after 3rd dose. But our study done on post exposure individuals not on healthy volunteers.

- In a study conducted in MANITOBE by AOKI FY et al on 24 healthy volunteers using human diploid cell vaccine along with immunoglobulin, the vaccines were given as 5 doses on day 0, 3, 7, 14 and 28. They all develop adequate antibodies after 3rd dose and no increase in titer was observed after the 4th and 5th dose. This was contrast to our study because the increase in antibody titer between third and fourth dose was significant.

- In a study conducted in MANITOBE by AKOI FY et al on both children and adult bitten by suspected rabid animal using human diploid cell vaccine along with immunoglobulin, the vaccines were given as 5 doses on day 0, 3, 7, 14 and 28. They all develop adequate antibodies after 3rd dose and no increase in titer was observed after the 4th and 5th dose. In contrast, our study result showed that even after 3rd dose adequate antibody levels were reached.

- In a study conducted in THAILAND by WASI.C et al on 27 children bitten by suspected rabid animals using purified chick embryo cell vaccine with or without immunoglobulin depending upon the class of wound, the vaccines were given as 6 doses on day 0, 3, 7, 14, 28 and 90. They all develop adequate antibodies after 3 doses. This correlates

with our study result. But there was no comparison with the fourth dose.

- In a study conducted in INDIA by BHATTACHARYA et al on 37 individuals bitten by suspected rabid animals using purified vero cell vaccine, 3 doses of vaccines were given on day 0, 3 and 7. They all develop adequate antibodies after 3 doses. This correlates with our study result. But there was no comparison with the fourth dose.

- In a study conducted in INDIA by BHATTACHARYA et al on 62 individuals bitten by suspected rabid animals using purified vero cell vaccine, 4 doses of vaccines were given on day 0, 3, 7 and 14. They all develop adequate antibodies after 4 doses. In contrast, our study result showed that even after 3rd dose adequate antibody levels were reached.

- in a study conducted in USA by JONES RL et al on 680 adult healthy volunteers using either human diploid cell vaccine or chromatographically purified rabies vaccine along with immunoglobulin, the vaccines were given as 5 doses on day 0, 3, 7, 14 and 28. All develop adequate antibodies on day 14. This correlates with our study result. But there was no comparison with the fourth dose.

- In a study conducted in WISCONSIN by LANG J et al on 32 adult healthy volunteers using either human diploid cell vaccine along with immunoglobulin, the vaccines were given as 5 doses on day 0, 3, 7, 14 and 28. All develop adequate antibodies on day 14. This correlates with our study result. But there was no comparison with the fourth dose.

- In a study conducted in THAILAND by BRIGGS DJ et al on 57 individuals presenting for post exposure prophylaxis using purified chick embryo cell vaccine with or without immunoglobulin depending upon the wound class, the vaccine were given as 6 doses on day 0, 3, 7, 14, 28 and 90. They all develop adequate antibody levels after 4 doses. In contrast, our study result showed that even after 3rd dose adequate antibody levels were reached.

- **In our study the rise in antibody titer when comparing the third dose with fourth dose is highly significant. Therefore fourth dose of anti-rabies vaccination should be given.**

- In a knowledge, attitude and practice study conducted in SRILANKA by GINO C MATIBAG et al regarding rabies found that knowledge

was adequate but attitude and health practice should be improves. This correlates with our study result.

- **In our study of knowledge attitude and practice regarding dog bite, knowledge was adequate but the attitude and practice were inadequate and should be improved.**

CONCLUSION

CONCLUSION

1. **In our study the rise in antibody titer when comparing the third dose with fourth dose is highly significant. Therefore fourth dose of anti-rabies vaccination should be given.**

2. **In our study of knowledge attitude and practice regarding dog bite, knowledge was adequate but the attitude and practice were inadequate and should be improved.**

LIMITATIONS

LIMITATIONS

- One limitation of this study is low number of patients. The results should be confirmed in study involving large number of patients.

- Long term follow up is required to assess the longevity of the anti-rabies antibody levels.

- Study should include the other approved anti-rabies vaccines for intramuscular route in assessing the antibody response.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Evidence for a 4-dose vaccine schedule for human rabies post-exposure prophylaxis in previously non-vaccinated individuals. Vaccine. 2009 Nov 27; 27(51):7141-8. doi: 10.1016/ j.vaccine.2009.09.029

2. Baer GM. Rabies: An historical perspective. Infect Agents Dis. 1994;3:168-180

3. Wunner WH. The chemical composition and molecular Structure of rabies viruses. In: Baer GM, Ed. The Natural History of Rabies. 2nd Ed. Boca Raton, Fla: CRC Press; 1991: 31-67.

4. Sokol F, Schlumberger HD, Wiktor TK, et al. Biochemical and biophysical studies on the nucleocapsid and on the RNA of Rabies virus. Virology. 1969; 38:651-665.

5. Dietzschold B, Rupprecht CE, Fu ZF, et al. Rhabdoviruses. In: Fields BN, Knipe DM, Howley PM, et al, eds. Fields Virology. 3rd ed. Philadelphia: Lippincott Raven; 1996:1137-1159.

6. Tordo N, Poch O, Ermine A, et al. Walking along the rabies Genome: Is the large G-L intergenic region a remnant gene? Proc Natl Acad Sci U S A. 1986; 83:3914-3918.

7. Bleck TP, Rupprecht CE. Rhabdoviruses. In: Richman DD, Whitley RJ, Hayden FG, eds. Clinical Virology. 2nd Ed. Washington, DC: ASM Press; 2002:857-873.

8. Yang J, Koprowski H, Dietzschold B, et al. Phosphorylation of Rabies virus nucleoprotein regulates viral RNA transcription and Replication by modulating leader RNA encapsidation. J Virol. 1999; 73:1661-1664.

9. Wu X, Gong X, Foley HD, et al. Both viral transcription and Replication are reduced when the rabies virus nucleoprotein is Not phosphorylated. J Virol. 2002; 76:4153-4161.

10. Goto H, Minimoto N, Ito H, et al. Expression of the nucleoprotein of rabies virus in Escherichia coliand mapping of antigenic Sites. Arch Virol. 1995; 140:1061-1074.

11. Blumberg BM, Giorgi C, Kolakofsky D. N protein of vesicular Stomatitis virus selectively encapsidates leader RNA in vitro. Cell. 1983; 32:559-567.

12. Tordo N, Poch O, Ermine A, et al. Completion of the rabies virus Genome sequence determination: Highly conserved domains Among the L (polymerase) proteins of unsegmentednegativestrand RNA viruses. Virology. 1988; 165:565-576.

13. Levy JA, Fraenkel-Conrat H, Owens RA. Virology. Englewood Cliffs, NJ: Prentice Hall; 1994:77-85.

14. Coll JM. The glycoprotein G of rhabdoviruses. Arch Virol. 1995; 140:827-851.

15. Mebatsion T, Weiland F, Conzelmann KK. Matrix protein of rabies virus is responsible for the assembly and budding of Bullet-shaped particles and interacts with the transmembrane Spike glycoprotein G. J Virol. 1999; 73:242-250.

16. Finke S, Mueller-Waldeck R, Conzelmann KK. Rabies virus Matrix protein regulates the balance of virus transcription and Replication. J Gen Virol. 2003; 84:1613-1621.

17. Finke S, Conzelmann KK. Dissociation of rabies virus matrix Protein functions in regulation of viral RNA synthesis and virus Assembly. J Virol. 2003; 77:12074-12082.

18. Rupprecht CE, Dietzschold B, Wunner WH, et al. Antigenic relationships of lyssaviruses. In: Baer GM, Ed. The Natural History Of Rabies. 2nd ed. Boca Raton, Fla: CRC Press; 1991:69-100.

19. Seif I, Coulon P, Rollin PE, et al. Rabies virulence: Effect on pathogenicity and sequence characterization of rabies virus Mutations affecting antigenic site III of the glycoprotein. J Virol. 1985; 53:926-934.

20. Morimoto K, Ni Y-J, Kawai A. Syncytium formation is induced In the murine neuroblastoma cell cultures which produce Pathogenic G proteins of the rabies virus. Virology. 1992; 189: 203-216.

21. Otvos L, Krivulka GR, Urge L, et al. Comparison of the effects Of amino acid substitutions and the β-N- vs. α-O-glycosylation On the T-cell stimulatory activity and conformation of an Epitope on the rabies virus glycoprotein. Biochim Biophys Acta. 1995; 1267:55-64.

22. Adapted from Rupprecht CE, Smith JS, Fekadu M, Childs JE. The ascension of wildlife rabies: A cause for public health concern or intervention? Emerg Infect Dis. 1995; 1:107-114.

23. Jackson AC. Human disease. In: Jackson AC, Wunner AH, eds. Rabies, 2nd edn. London: Elsevier, Academic Press; 2007:309–40.

24. Helmick CG, Tauxe RV, Vernon AA. Is there a risk to contacts of patient's withrabies? Rev Infect Dis 1987; 9:511–18

25. Finke S, Conzelmann KK. Dissociation of rabies virus matrix Protein functions in regulation of viral RNA synthesis and virus Assembly. J Virol. 2003; 77:12074-12082

26. Murphy FA, Bauer SP, Harrison AK, et al. Comparative pathogenesis of rabies and rabies-like viruses: Virus infection and transit from inoculation site to the central nervous system. Lab Invest. 1973; 28:361-376.

27. Watson HD, Tignor GH, Smith AL. Entry of rabies virus into The peripheral nerves of mice. J Gen Virol. 1981; 56:371-382.

28. Lentz TL, Barrage TG, Smith AL, et al. Is the acetylcholine receptor a rabies virus receptor? Science. 1982; 215:182-184.

29. Lentz TL, Wilson PT, Hawrot E, et al. Amino acid sequence Similarity between rabies virus glycoprotein and snake venom Curare mimetic neurotoxins. Science. 1984; 226:847-848.

30. Kelly RM, Strick PL. Rabies as a transneuronal tracer of circuits In the central nervous system. J Neurosci Methods. 2000; 103: 63-71

31. Esiri MM, Kennedy PGE. Virus diseases. In: Adams JH, Duchen LW, eds. Greenfield's Neuropathology. New York: Oxford University Press; 1992:335-399

32. De Brito T, Araujo MD, Tiriba A. Ultrastructure of the Negri Body in human rabies. J Neurol Sci. 1973; 20:363-372.

33. Fishbein DB, Bernard KW. Rabies virus. In: Mandell GM, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases. New York: Churchill Livingstone; 1994:1527-1543.

34. Hemachuda T, Phanthumchinda K, Phanuphak P, et al. Myoedema as a clinical sign in paralytic rabies. Lancet. 1987; 1:1210.

35. Gowers WR. A manual of diseases of the nervous system. Philadelphia: Blakiston; 1888:1237-1254.

36. Warrell DA, Davidson NM, Pope HM, et al. Pathophysiologic Studies in human rabies. Am J Med. 1976; 60:180-190?

37. Cheetham HD, Hart J, Coghill NF, et al. Rabies with myocarditis: Two cases in England. Lancet. 1970; 1:921-922

38. Blenden DC, Creech W, Torres-Anjel MJ. Use of immune fluorescence examination to detect rabies virus in the skin of Humans with clinical encephalitis. J Infect Dis. 1986; 154: 698-701.

39. Crepin P, Audry L, Rotivel Y, et al. Intravital diagnosis of human Rabies by PCR using saliva and cerebrospinal fluid. J Clan Microbiol. 1998; 36:1117-1121.

40. Arai YT, Yamada K, Kameoka Y, et al. Nucleoprotein gene analysis of fixed and street rabies virus variants using RT-PCR. Arch Virol. 1997; 142:1787-1796.

41. Nadin-Davis SA. Polymerase chain reaction protocols for rabiesvirus discrimination. J Virol Methods. 1998; 75:1-8

42. Whitby JE, Johnstone P, Sillero-Zubiri C. Rabies virus in the Decomposed brain of an Ethiopian wolf detected by nested Reverse transcription-polymerase chain reaction. J Wildl Dis. 1997; 33:912-915.

43. Albas A, Ferrari CI, da Silva LH, et al. Influence of canine brain Decomposition on laboratory diagnosis of rabies. Rev Soc Bras Med Trop. 1999; 32:19-22.

44. Zaidman GW, Billingsley A. Corneal impression test for the Diagnosis of acute rabies encephalitis. Ophthalmology. 1998; 105: 249-251.

45. Smith JS, Yager PA, Baer GM. A rapid reproducible test for determining rabies neutralizing antibody. Bull WHO. 1973; 48:535-541.

46. Alvarez L, Fajardo R, Lopez E, et al. Partial recovery from rabies in a nine-year-old boy. Ped Infect Dis J. 1994;13:1154-1155.

47. Laothamatas J, Hemachuda T, Mitrabhakdi E, et al. MR imaging In human rabies. AJNR Am J Neuroradiology. 2003; 24:1102-1109

48. National Guidelines on Rabies Prophylaxis 2013

49. Warrell MJ. Current rabies vaccines and prophylaxis schedules: preventing rabies before and after exposure. Travel Med INF Dis 2012 (in press)doi:10.1016/j.tmaid.2011.12.005.

50. Wiktor TJ, Doherty PC, Koprowski H. In vitro evidence of cell-mediated immunity after exposure of mice to both live and inactivated rabies virus. Proceedings of the National Academy of Sciences of the United States of America, 1977, 74:334–338.

51. Hooper C et al. Collaboration of antibody and inflammation in clearance of rabies virus from the central nervous system. Journal of Virology, 1998, 72:3711–3719.

52. Moore SM et al. Detection of cellular immunity to rabies antigens in human vaccines. Journal of Clinical Immunology, 2006, 26:533–545.

53. Brinkman DM et al. Vaccination with rabies to study the humoral and cellular immune response to a T-cell dependent neoantigen in man. Journal of Clinical Immunology, 2003, 23:528–538.

54 .Shankar V, Dietzschold B, Koprowski H. Direct entry of rabies virus into the central nervous system without prior local replication. Journal of Virology, 1991, 65:2736–2738.

55. Herzog M et al. Nucleocapsid specific T and B cell responses in humans after rabies vaccination. Virus Research, 1992, 24:77–89.

56. WHO position paper on rabies vaccines? Weekly Epidemiological Record, 2010, 85 (31):293–308.

57. WHO Expert Consultation on Rabies. First Report. Ge n e v a, World Health Organization, 2004 (WHO Technical Report Series, No. 931).

58.Rabies. Scientific Basis of the Disease and Its Management (2013) Alan C.Jackson

59. Clin Infect Dis. (2003) 36 (1): 60-63.doi: 10.1086/344905 http://cid.oxfordjournals.org/content/36/1/60.full

ANNEXURES

PROFORMA

NAME OF THE PATIENT :

AGE / SEX :

IP/OP NUMBER :

OCCUPATION :

ADDRESS :

CONTACT NUMBER :

COMPLAINTS :

PAST HISTORY :

TREATMENT HISTORY :

DRUG ALLERGY :

GENERAL EXAMINATION :

VITALS :

LOCAL EAMINATION OF BITE SITE:

SYSTEMIC EXAMINATION

CENTRAL NERVOUS SYSTEM:

CARDIOVASCULAR SYSTEM:

RESPIRATORY SYSTEM :

ABDOMEN :

DATE OF FIRST DOSE OF ARV:

SERUM ANTI RABIES ANTIBODY TITERS

ON 14TH DAY AFTER FIRST DOSE OF ARV [7 DAYS AFTER 3RD DOSE] :

ON 21ST DAY AFTER FIRST DOSE OF ARV [7 DAYS AFTER 4TH DOSE] :

COMPLIANCE :

KNOWLEDGE ATTITUDE AND PRACTICES REGARDING DOG BITE IN PATIENTS COMING FOR POST EXPOSURE PROPHYLAXIS

1. What treatment options are available for dog bite?

2. What is mode of transmission of rabies?

3. How many days one should observe the dog following bite? And what for?

4. Your opinion regarding wound washing and immediate wound suturing?

5. Chances of survival if rabies develops?

6. Your opinion regarding prophylaxis for provoked dog bite?

7. Whether all doses of ARV In the schedule should be completed?

8. Your opinion whether ARV needed in vaccinated dog bites?

9. Whether avoidance of certain foods needed after dog bite for any particular period?

10. Will rabies spread from human to human?

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr.A.Abinantha Raju,
Post Graduate, MD (General Medicine),
Institute of Internal Medicine,
Madras Medical College,
Chennai – 600 003.

Dr.A.Abinantha Raju,

The Institutional Ethics Committee has considered your request and approved your study titled **"Comparison of antibody titers between third and fourth IM dose of anti rabies vaccination following post exposure prophylaxis with anti rabies immunoglobulin and KAP (Knowledge Attitute and Practice) among patients coming for post exposure prophylaxis of dog bite"** No.49072014.

The following members of Ethics Committee were present in the meeting held on 01.07.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5. Dr.G.Muralidharan, Director Incharge, Inst.of Surgery	: Member
6. Prof.Md.Ali, M.D., D.M., Prof & HOD of MGE, MMC	: Member
7. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
8. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
9. Prof.Tito, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
10. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
11. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
12. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

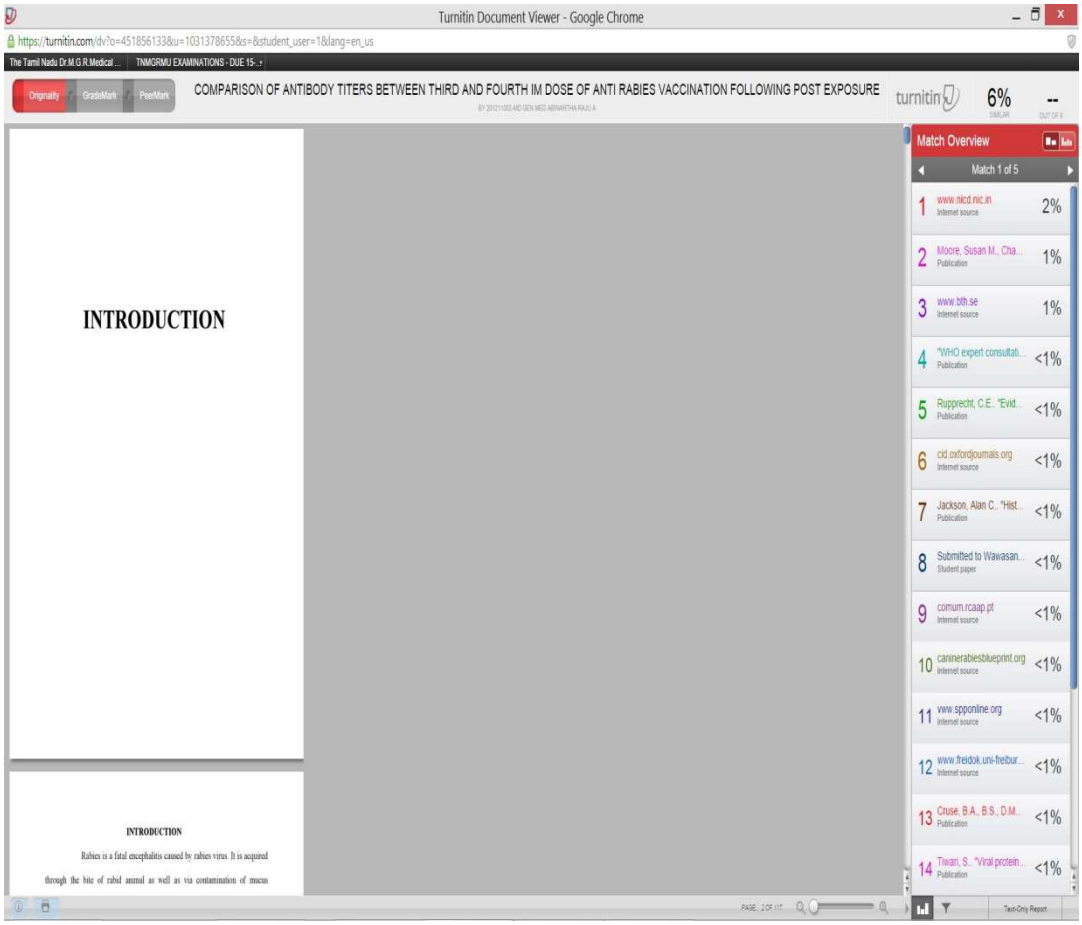
Sd/ Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

TURNITIN PLAGIARISM SCREEN SHOT



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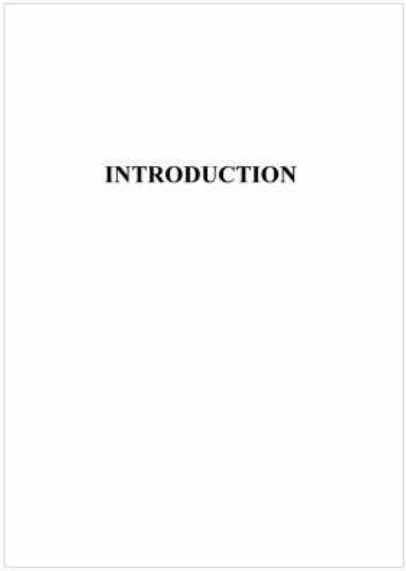

INTRODUCTION

INFORMATION SHEET

We are conducting a study on **"COMPARISON OF ANTIBODY TITERS BETWEEN THIRD AND FOURTH IM [INTRAMUSCULAR] DOSE OF ANTI RABIES VACCINATION FOLLOWING POST EXPOSURE PROPHYLAXIS WITH ANTI-RABIES IMMUNOGLOBULIN, AND KAP [KNOWLEDGE ATTITUTE AND PRACTICE] AMONG PATIENTS COMING FOR POST EXPOSURE PROPHYLAXIS OF DOG BITE"** among patients attending Rajiv Gandhi Government General Hospital, Chennai and for that your specimen may be valuable to us.

The purpose of this study is to assess the **"COMPARISON OF ANTIBODY TITERS BETWEEN THIRD AND FOURTH IM [INTRAMUSCULAR] DOSE OF ANTI RABIES VACCINATION FOLLOWING POST EXPOSURE PROPHYLAXIS WITH ANTI-RABIES IMMUNOGLOBULIN, AND KAP [KNOWLEDGE ATTITUTE AND PRACTICE] AMONG PATIENTS COMING FOR POST EXPOSURE PROPHYLAXIS OF DOG BITE"**

We are selecting certain cases and if you are found eligible, we may be using your specimen to perform extra tests and special studies which in any way do not affect your final report or management.

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator | Signature of Participant

PATIENT CONSENT FORM

Study Title : **"COMPARISON OF ANTIBODY TITERS BETWEEN THIRD AND FOURTH IM [INTRAMUSCULAR] DOSE OF ANTI RABIES VACCINATION FOLLOWING POST EXPOSURE PROPHYLAXIS WITH ANTI-RABIES IMMUNOGLOBULIN, AND KAP [KNOWLEDGE ATTITUTE AND PRACTICE] AMONG PATIENTS COMING FOR POST EXPOSURE PROPHYLAXIS OF DOG BITE"**

Study Centre : Rajiv Gandhi Government General Hospital, Chennai.

Name :

Age/Sex :

Identification Number :

Patient may check (☑) these boxes

The details of the study have been provided to me in writing and explained to me in my own language	❑
I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected.	❑
I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study.	❑
I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms.	❑
I hereby consent to participate in this study.	❑
I hereby give permission to undergo complete clinical examination, diagnostic tests including hematological, biochemical tests and radiological tests.	❑

Signature/ Thumb impression

Signature of the investigator

Patient's name and address

Study Investigator's name

Dr. A.ABINANTHA RAJU

MASTER CHART

S.No	AGE	SEX	14th DAY RFFIT VALUE	21ST DAY RFFIT VALUES	KNOWLEDGE Q1	KNOWLEDGE Q2	KNOWLEDGE Q3	KNOWLEDGE Q4	KNOWLEDGE Q5	ATTITUDE PRACTICE Q1	ATTITUDE PRACTICE Q2	ATTITUTE PRACTICE Q3	ATTITUDE PRACTICE Q4	ATTITUDE PRACTICE Q5
1	50	M	197.5	216.23	1	1	1	1	0	1	0	1	0	0
2	44	M	150.26	180.25	1	1	1	1	0	1	0	1	0	0
3	48	F	175.68	198.36	1	1	1	1	0	1	0	1	0	0
4	45	M	181.47	199.65	1	1	1	1	0	1	0	1	0	0
5	62	M	194.85	220.64	1	1	1	1	0	1	0	1	0	0
6	16	M	184.89	193.63	1	1	1	1	0	1	1	1	1	0
7	25	M	196.57	226.11	1	1	1	1	0	1	0	1	0	0
8	65	M	176.24	193.26	1	1	1	1	0	1	0	1	0	0
9	48	M	189.45	206.13	1	1	1	1	0	1	0	0	0	0
10	35	M	166.74	195.2	1	1	1	1	0	1	1	0	1	0
11	36	M	169.68	194.58	1	1	1	1	0	1	0	0	0	0
12	66	M	173.25	206.03	1	1	1	1	0	1	0	0	0	0
13	44	M	184.37	215.34	1	1	1	1	0	1	0	0	0	0
14	52	M	210.55	220.35	1	1	1	1	0	1	0	1	0	0
15	19	M	203.56	221.54	1	1	1	1	0	1	0	1	0	0
16	54	M	184.36	199.33	1	1	1	1	0	1	0	1	0	0
17	15	F	189.37	216.42	1	1	1	0	1	1	0	1	0	0
18	36	F	178.35	204.03	1	1	1	1	0	1	1	1	1	0
19	63	M	196.33	215.39	1	1	1	1	0	1	0	1	0	0

20	44	F	183.2	213.06	1	1	1	1	0	1	1	1	1	0
21	51	F	162.08	194.17	1	1	1	1	0	1	0	1	0	0
22	62	F	178.26	201.25	1	1	1	1	0	1	1	1	1	0
23	35	M	177.02	213.05	1	1	1	1	0	1	0	0	0	0
24	26	M	189.05	210.32	1	1	1	1	0	1	1	0	1	0
25	14	M	198.33	224.06	1	1	1	1	0	1	1	0	1	0
26	56	M	186.06	214.36	1	1	1	1	0	1	1	0	1	0
27	23	M	194.51	223.04	1	1	1	1	1	1	1	0	1	0
28	26	M	162.89	183.02	1	1	1	1	0	1	1	1	1	0
29	45	M	172.08	200.36	1	1	1	1	0	1	0	1	0	0
30	65	M	163.24	189.49	1	1	1	0	0	1	0	1	0	0
31	45	M	188.42	216.48	1	1	1	0	0	1	0	0	0	0
32	63	M	163.24	198.55	1	1	1	1	0	1	1	0	1	0
33	32	M	178.26	204.36	1	1	1	1	0	1	1	0	1	0
34	39	M	184.33	214.77	1	1	1	1	0	1	1	0	1	0
35	65	M	162.48	186.48	1	1	1	1	0	1	1	0	1	0
36	24	M	172.22	194.89	1	1	1	0	0	1	1	1	1	0
37	45	M	193.44	211.68	1	1	1	0	0	1	0	1	0	0
38	36	M	186.48	198.14	1	1	1	0	0	1	0	1	0	0
39	26	M	166.89	176.25	1	1	1	1	0	1	0	1	0	0
40	32	M	156.33	186.79	1	1	1	1	0	1	0	1	0	0
41	54	M	148.26	173.93	1	1	1	1	1	1	0	1	0	0
42	55	F	163.78	177.58	1	1	1	1	1	1	0	1	0	0
43	66	F	163.55	186.34	1	1	1	0	0	1	0	1	0	0
44	62	F	153.26	199.06	1	1	1	1	0	1	0	1	0	0
45	53	M	188.49	221.25	1	1	1	1	1	1	0	1	0	0
46	43	M	146.78	194.27	1	1	1	1	0	1	0	1	0	0

47	65	M	186.33	206.91	1	1	1	1	1	1	1	1	1	0
48	15	M	168.29	189.04	1	1	1	1	0	1	0	1	0	0
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50	45	F	186.47	206.46	1	1	1	1	0	1	0	0	0	0
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52	49	M	196.35	216.21	1	1	1	1	0	1	0	0	0	0
53	68	F	146.25	186.34	1	1	1	1	0	1	0	0	0	0
54	47	F	169.77	179.03	1	1	1	1	0	1	0	0	0	0
55	56	M	158.26	198.04	1	1	1	1	0	1	0	0	0	0
56	36	M	154.39	173.29	1	1	1	1	0	1	1	0	1	0
57	59	M	163.48	196.28	1	1	1	1	0	1	0	0	0	0
58	62	M	148.33	183.26	1	1	1	1	0	1	0	1	0	0
59	53	M	186.49	203.15	1	1	1	1	0	1	0	1	0	0
60	59	M	166.03	198.35	1	1	1	1	0	1	0	1	0	0
61	65	M	155.98	184.26	1	1	1	1	0	1	0	1	0	0
62	59	M	184.26	207.65	1	1	1	1	0	1	0	1	0	0
63	15	M	145.06	198.34	1	1	1	1	0	1	1	1	1	0
64	26	M	213.05	218.35	1	1	1	1	0	1	0	0	0	0
65	32	M	216.36	224.16	1	1	1	1	0	1	0	0	0	0
66	58	F	186.24	201.49	1	1	1	1	0	1	0	0	0	0
67	49	F	166.36	189.24	1	1	1	1	0	1	0	0	0	0
68	65	F	189.63	196.78	1	1	1	1	0	1	0	0	0	0
69	58	M	146.22	173.25	1	1	1	1	0	1	1	0	1	0
70	56	F	169.04	196.27	1	1	1	1	1	1	0	0	0	0
71	49	F	178.22	201.56	1	1	1	1	0	1	0	1	0	0
72	43	F	156.08	178.33	1	1	1	1	1	1	0	1	0	0
73	48	M	174.32	196.27	1	1	1	1	1	1	0	1	0	0

74	15	M	186.06	201.56	1	1	1	1	0	1	1	1	1	0
75	68	M	198.35	213.89	1	1	1	1	1	1	0	0	0	0
76	43	F	163.28	198.24	1	1	1	1	0	1	0	0	0	0
77	29	M	145.32	183.21	1	1	1	1	0	1	0	0	0	0
78	65	F	198.26	221.45	1	1	1	1	0	1	0	0	0	0
79	26	M	186.24	201.65	1	1	1	1	0	1	0	0	0	0
80	30	F	174.36	203.81	1	1	1	1	0	1	1	0	1	0
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82	19	M	189.32	204.21	1	1	1	1	1	1	0	0	0	0
83	64	M	146.89	186.22	1	1	1	1	0	1	0	0	0	0
84	28	F	166.24	194.26	1	1	1	1	0	1	1	0	1	0
85	26	M	189.64	205.68	1	1	1	1	0	1	0	0	0	0
86	39	M	198.65	226.35	1	1	1	1	0	1	0	1	0	0
87	37	M	201.35	224.3	1	1	1	1	0	1	0	1	0	0
88	15	M	176.54	189.23	1	1	1	1	0	1	0	1	0	0
89	41	M	178.66	192.03	1	1	1	1	0	1	0	1	0	0
90	26	F	186.47	196.43	1	1	1	1	0	1	0	1	0	0
91	24	F	153.26	186.05	1	1	1	1	0	1	0	0	0	0
92	53	F	148.33	197.02	1	1	1	1	0	1	0	0	0	0
93	68	F	198.32	201.56	1	1	1	1	0	1	0	0	0	0
94	53	M	168.24	196.24	1	1	1	1	0	1	0	0	0	0
95	54	M	163.49	198.32	1	1	1	1	1	1	0	0	0	0
96	24	M	165.23	175.03	1	1	1	1	1	1	0	0	0	0
97	56	M	149.79	163.04	1	1	1	1	0	1	0	0	0	0
98	24	M	168.94	193.06	1	1	1	1	0	1	0	0	0	0
99	35	M	198.32	213.58	1	1	1	1	0	1	0	0	0	0
100	41	M	186.24	221.38	1	1	1	1	0	1	0	0	0	0